EVALUATION OF A STERILE PULPOTOMY PROCEDURE

by

James R. Roche

Submitted to the Faculty of the Graduate School in partial fulfillment of the requirements for the degree of Master of Science in Dentistry, Indiana University School of Dentistry, 1983.

ACKNOWLEDGMENTS

The author expresses great appreciation to the members of his Graduate Committee for their guidance with this thesis investigation. Dean Ralph E. McDonald is recognized for his scholarly counsel during the course of this research, his masterly teaching, and his long-standing friendship. Special thanks are extended to Professor Paul Barton for his expert editing, positive spirit, and patient direction of the manuscript.

Sincere appreciation is offered to Dr. Abdel H. Kafrawy for sharing his scholarly insights and judgment about research design, and for his meticulous analysis of the histologic sections. Gratitude is also expressed to Dr. Chris H. Miller for his generous sharing of microbiologic applications and his strong interest in the study.

Dr. Meryl Englander is held in high esteem as a respected colleague, and he is gratefully acknowledged for his ability to communicate his expertise in finding the key question in any issue.

Special gratitude goes to Mrs. Penny Mead for her effective dental assisting during the surgical procedures; to Miss Shirley Shazer for preparing the histologic sections; to Mrs. Karen Gremore for her expert typing of the manuscript and devotion to the project; to Mr. Richard Scott and his staff for helping with the photographs; and to Dr. Leonard G. Koerber for his able assistance in preparing the statistical analysis for this study.

The author expresses special thanks to his wife, Vi, for her love, faith, and perpetual encouragement in the development of this research, as well as for her attentive assistance as a nurse in the sterile surgery. Mr. George O. Morris is recognized for his faith in this investigation, and for his love as a father-in-law. Very noteworthy are the steadfast love and confidence given by the author's daughter, Ann, his son-in-law, Steve, and granddaughters Allison Marie and Kelly Ann.

From all of these friends and loved ones, along with many others, have come the support and inspiration that kindle thought and stimulate a determination to seek new scientific achievement.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

INTRODUCTION

Pulpotomy procedures for the treatment of pulpal exposure or pulpal disease have traditionally used an approach from the occlusal surface in primary molars, with a rotating round bur or a small spoon excavator being used to amputate the pulp under clean conditions. Also, in almost all animal and human studies a medicament has been applied to the amputation site.

The purpose of this study was: (1) to determine the feasibility of performing a sterile scalpel excision of coronal pulpal tissue, and (2) to evaluate wound healing after a shield has been placed to prevent all substances from touching the blood clot at the amputation site.

Hypotheses for this investigation were as follows:

1. The surface of a dog's tooth can be rendered free of cultivable bacteria and the operative site maintained in this condition throughout pulpotomy procedures.
2. The dentin from the buccal surface of the tooth can be removed to expose the coronal portion of the pulp without grossly macerating the pulpal tissue.
3. A scalpel severance of the pulpal tissue in dogs can be performed so that the coronal portion of the pulp will be removed from the pulp chamber.
4. A diaphragm can be constructed under sterile operating room conditions, applied to the cavity preparation, and secured in place without touching the pulpal amputation site.

5. The diaphragm can be secured in place for a convalescent period of at least three weeks.
6. After a convalescent period of three weeks, there can be satisfactory wound healing in the pulpal tissue, as manifested by the absence of inflammatory cells, or by the presence of only a moderate or mild inflammatory infiltrate that gives evidence of being reversible.

A series of five experiments was conducted to support the research design of the main study. These trials included testing a tissue-protecting device to remove the buccal dentin over pulp tissue, amputating the pulp with a scalpel, using antimicrobial agents on the tooth surface, and reducing the possibility of bacteria invading the pulpal amputation site from the use of rotary instruments and from leakage at the cavity seal.

REVIEW OF LITERATURE

This review of literature is presented under the following headings: Disinfection of Enamel Surfaces, Pulpotomy Techniques, Irrigating Solutions, Hemostasis, Marginal Leakage, Wound Healing, and Immunology.

Disinfection of Enamel Surfaces

Wunder and associates[1] identified 15 different kinds of microorganisms in dental plaque of beagle dogs, including 11 types of gram-positive bacteria and four of gram-negative microorganisms. Streptococci represented only a small portion of the total plaque flora. Courant and associates[2] reported on the total bacteria count in the oral flora of beagle dogs. Samples of gingival crevice debris revealed that approximately three and one-half times more bacteria were associated with periodontal pockets than with healthy gingival sulci.

Birch and Melville[3] reported effective disinfection of enamel surfaces in human subjects with the use of tincture of iodine or a 0.5 percent of Hibitane solution. The solutions were permitted to act on the enamel surface for three minutes. In the trials with iodine, 4.7 percent of the cultures were positive for the presence of microorganisms and 6.2 percent of the samples were positive when Hibitane was used.

In testing the efficacy of cleansing agents and antimicrobial agents on 20 rubber dam pieces coated with spore-containing bacteria, Moller[4] demonstrated a consistent freedom from cultivable bacteria after the items were placed in a cleansing solution of 30 percent

hydrogen peroxide for one minute and then immersed in 5 percent tincture of iodine for 15 minutes. In addition, he obtained disinfection of 19 test samples of human enamel in a group of 20, which were debris-coated and infected with a spore-free culture, by immersing the enamel pieces in 30 percent hydrogen peroxide for three minutes and then immersing them in 5 percent tincture of iodine for one minute. Moller also infected debris-coated enamel pieces with a spore-free culture and applied glycerin as a lubricant before permitting 5 percent tincture of iodine to contact the contaminated specimens for 16 minutes without cleansing with hydrogen peroxide. No test pieces were free of cultivable microorganisms.

Moller[4] also compared the sterilization of 355 tooth surfaces in 336 patients by two methods: one using hydrogen peroxide and tincture of iodine, and the other using tincture of iodine alone. In the first group, 98 percent of the tooth surfaces were free of cultivable bacteria, and in the second group, using the rubber dam and the iodine solution alone, only 41 percent of the surfaces were free of cultivable microorganisms. When the junction between the tooth and rubber dam was cultured, 83 percent of the cultures were negative for bacteria in the group using both hydrogen peroxide and tincture of iodine. In the group using iodine, only 6 percent of the cultures were negative for bacteria at the junction of the tooth and rubber dam.

In the same report, Moller demonstrated that 19 of 20 pieces of mucous membrane from newly slaughtered cattle, which had been infected with spore-free cultures, were disinfected by being immersed for one minute in 10 percent hydrogen peroxide and then immersed for one minute in 5 percent tincture of iodine.

In 1979 Griffee[5] used a technique similar to Moller's to disinfect tooth surfaces in a clinical study with successive applications of 30 percent hydrogen peroxide and 2 percent tincture of iodine. Cultures were not taken of the tooth surfaces, but clinical observations indicated successful disinfection.

In a laboratory study of 10 human teeth obtained immediately after extraction, Ahmed and Russell[6] demonstrated the value of mechanical cleaning of tooth surfaces before applying sterilization procedures. Scaling and polishing, together with a mechanical preparation and thorough washing of the root canals, reduced the bacterial population of the teeth by a factor of approximately 10^4. The anaerobic organisms appeared to be most affected by the mechanical cleaning.

In an _in vivo_ study, Melville and Birch[7] demonstrated the importance of thoroughly cleansing the tooth surface before applying a sterile rubber dam to secure an endodontic field free of cultivable bacteria. A significant decrease in the number of positive cultures resulted when study teeth were polished for one and one-half minutes with a prophylactic paste before applying a sterile rubber dam and painting the rubber dam field and exposed teeth with a 0.1 percent solution of acriflavine.

Other investigations involving efforts to disinfect enamel in preparation for pulpotomy procedures include a study by Armstrong et al[8]. After rubber dams were applied to 40 monkey teeth in preparation for pulpotomy procedures, the operating fields were swabbed with merthiolate. However, no evaluation was made of the operating fields for cultivable bacteria, and the contaminating effect of operating high and low speed handpieces on the preparations was not determined.

In 1971 Schroder and Granath[9] used a clean pulpotomy technique with sterile instruments by applying rubber dam material to eight mandibular second primary molar teeth, removing the gross dental caries, and then cleaning the field of operation with 3 percent hydrogen peroxide and alcohol. No measure of the disinfection capability was obtained.

Magnusson[10] performed pulpotomies on 100 mandibular primary molar teeth in children 4 to 9 years of age, after applying rubber dam material and washing the operative field with a solution of quaternary ammonium compound. No attempts were made to evaluate the enamel surfaces for cultivable bacteria before excising the coronal pulps. However, when bacteriologic samples of the pulpal wounds in distal canals were cultured for 60 of these teeth after pulpal amputation, 29 gave negative bacteriologic cultures and 31 demonstrated bacteria. A calcium hydroxide paste was applied to the amputation sites. No correlation could be demonstrated between the results of the bacterial cultures and the dominant histologic finding of inflammation of the residual pulp tissue and internal root resorption.

In performing partial pulpectomies on 23 permanent teeth, Engstrom and Spangberg[11] applied rubber dams and then washed the operating field with hydrogen peroxide and 5 percent iodine in alcohol. Bacteriologic specimens were obtained at the time of pulpal amputation. Both Brewer's thioglycollate and brain-liver-heart media showed negative growth for all cultures.

Several other reports[12-15] of pulpotomy procedures have described a variety of attempts to disinfect the enamel surface before entering the tooth, but the effectiveness of the disinfecting procedures has not

been measured. In addition, many reports of different pulpotomy procedures[16-24] have included no mention of efforts to disinfect the enamel surface.

Bergenholtz[25] demonstrated in monkey teeth that products of bacteria applied to exposed dentin can initiate significant inflammatory reactions in the dental pulp. Brown[26] showed that an increased bacterial aerosol is created when ultra high-speed cutting instruments are used. He recommended that dentists wear surgical masks while performing operative dentistry and that teeth receiving treatment be mechanically cleaned, isolated with rubber dam material, and their enamel surfaces disinfected.

Pulpotomy Techniques

In 1948 Kreshover and Bevelander[27] studied pulpal responses in 14 dog teeth after exposing the pulps with a small round bur. Attempts were made to maintain aseptic conditions with the use of rubber dam. Following pulp exposure, a disc of tinfoil was placed on the cavity floor and the preparation was sealed with gutta-percha or oxyphosphate of zinc cement. Histologic sections prepared 7 to 47 days after the operation revealed gradations of inflammatory responses, such as abscess formation and massive round cell infiltration in the coronal pulp with normal-appearing odontoblasts in the radicular portion. Later sections demonstrated granulation tissue which proceeded to fibrosis. An osteodentin-like formation surrounded spicules of dentin which were inadvertently thrust into the pulp during the exposure of pulpal tissue.

Despite differences between the design of the present thesis investigation and the study by Kreshover and Bevelander (such as their failure to maintain a sterile operating field and the fact that gutta-percha and oxyphosphate of zinc cement were used to seal the preparation, which raises doubts as to whether there was microleakage of microorganisms at the interface of the temporary restoration), that earlier study was similar to this investigation in that a surgical procedure was performed on the pulp in an attempted aseptic field of operation and no medicament was applied directly to the excised pulpal tissue.

Granath and Hagman[28] performed an experimental pulpotomy on nine mandibular bicuspid teeth for patients receiving orthodontic treatment. After a local anesthetic was administered and a rubber dam was applied to the experimental tooth, the operating field was cleaned with 30 percent hydrogen peroxide and absolute alcohol and washed with 10 percent tincture of iodine. All instruments and materials were sterilized, but no mention was made as to whether a sterile operating technique was followed. Diamond instruments were used to remove the occlusal enamel and dentin to form a cylindrical well preparation. A coronal portion of the pulp was removed by a cylindrical diamond stone. During the operative procedures, the tooth was irrigated with physiologic saline solution. A high-speed handpiece (100,000 rpm.) was used for the inital enamel preparation and removal of pulp tissue. The well in the dentin was cut with a low-speed handpiece at intermittent pressure.

After a portion of the coronal pulp had been ground away, the pulpal stump was continuously irrigated with physiologic saline solution until bleeding ceased. A 0.2 millimeter thick disc of Teflon was placed on the dentin shelf and secured in place with a low melting-point wax. Pharmatic cement was placed over the wax and the cavity was restored with zinc phosphate cement.

The pulps of two teeth that were extracted immediately after physiologic hemostasis showed very little damage, with unaltered odontoblastic layers. Two teeth extracted 24 hours after physiologic hemostasis demonstrated intra-pulpal hemorrhage extending from the surface to a depth of 3 millimeters, and granulocytes and some lymphocytes were present in the area. Histologic sections of the four teeth extracted after four weeks revealed mild inflammatory changes (i.e. dilated blood vessels and an increased number of histiocytes in deeper areas). In the one case where the disc of Teflon was applied directly onto the bleeding pulp, the tooth was extracted after one week. This patient had experienced pain at bedtime and the tooth was tender while chewing during the entire week. Histologic sections showed degenerative changes with vacuolation in the odontoblastic layer and loss of cellular detail throughout the pulp.

In 1973 Schroder[29] evaluated pulpal healing histologically following pulpotomy treatment in 18 human mandibular bicuspids. Healthy pulpal tissue was amputated under aseptic conditions, using the technique developed by Granath and Hagman[28]. The pulpal wound was rinsed with a saturated sterile aqueous solution of calcium hydroxide.

A blood clot was permitted to form at the amputation site before the application of a sterile calcium hydroxide paste. Then the pulps were treated in the manner described by Schroder and Granath[30]. The experimental teeth were extracted between two weeks and one month after receiving a pulpotomy. Although the restorative material used was not identified, it is assumed that it was zinc phosphate cement, as in the method of Granath and Hagman[28]. The results of this study indicated that an extra-pulpal blood clot between the wound surface and the calcium hydroxide interfered with pulpal healing. If the criterion for complete healing is considered to be the development of a continuous hard tissue barrier, with a residual pulp demonstrating no inflammatory infiltrate, four teeth (22%) showed healing. One case was considered questionable and healing was non-existent in 13 cases. The apical portion of the pulp was always of normal appearance. There was no mention of possible microleakage of bacteria at the interface of the zinc phosphate cement restoration and the cavity preparation.

In 1921 Davis[31, 32] expressed his belief (with references to clinical cases) that residual pulp tissue amputated at some level between the pulp chamber and the apex will remain vital. He added that the remaining pulp will achieve desirable root canal obliteration with osteoid tissue, in the absence of gross infection and destructive drugs. Based on Grove's[33] dental anatomy studies which indicated the difficulty of removing all pulpal tissue from multi-canals in the apical region for pulpectomy treatment, Davis[34] in 1922 continued to recommend pulpotomy treatment for vital pulps. He stated that if the pulpal tissue is not overwhelmed by bacteria, the pulp has a better opportunity than other tissues to repair in an orderly manner.

Principal features of the first phase of Davis' pulpotomy technique were: (1) Avoid infection and all drugs that might injure the pulp or impair circulation, (2) apply rubber dam, (3) mechanically and chemically sterilize the immediate and surrounding operating field, (4) boil all instruments, (5) use a pulp knife, (6) check pulpal hemorrhage with hot water or use Adrenalin cautiously, (7) apply a dressing of phenolized eugenoe or camphophenique. The second stage, after 6 to 24 hours, included these steps: (1) Apply the rubber dam, (2) sterilize the operating field, (3) remove the dressing and irrigate the canal with 50 to 60 percent alcohol, (4) wash the canal with boiled water, (5) place a fine orthodontic wire (which has been previously passed through a flame) in the canal to the level of the pulpal stump (which will be determined by the patient's flinching) and take care not to injure the pulp again.

If the pulpal amputation was located deep within the canal, a varnish and a gutta-percha point were placed in the canal. If the pulp was excised at the floor of the pulp chamber, a dressing of phenol, eugenoe, plaster of paris, and zinc sulphate was applied to the amputation site. Davis felt that the best level of pulpal amputation was open to question, but said that the type of pulpal dressing used did not seem to make any difference as long as the principles of tissue repair were not violated. No histologic studies were presented.

Subsequently, Davis[35] presented microscopic sections of cases showing two responses to his pulpotomy technique. One reaction was the persistence of vitality in the residual pulpal tissue with a deposition

of osteoid tissue about the pulp in concentric rings. The other response showed that the pulp canal had been completely filled with new osteoid tissue.

In a later paper during 1922, Davis[36] reported that only one percent of over 400 pulpotomy cases developed postoperative pericementitis. In the following year[37] he proposed that contrary to the treatment philosophy of that period, the pulp could tolerate surgical treatment and undergo repair in the same way as other connective tissues, if it was not overwhelmed by bacteria. He emphasized the need of using antiseptic precautions similar to those practiced in general surgery.

In 1963 Nyborg and Halling[38] histologically compared healthy pulpal stumps which were amputated with either an Antheos reamer or the cut-tip Hedstrom file. Thirteen contralateral pairs of human incisors and cuspids were treated, and six additional teeth were used as controls.

The pulp was exposed under aseptic conditions (use of rubber dam was not mentioned) and the superior portion of the pulp was removed with a round bur. The use of both instruments involved twisting the pulp in a clockwise direction. At random, the Antheos reamer was used on one side of the patient's mouth and the Hedstrom file on the other. The pulps were excised deep within the root canals (partial pulpectomy). In most of the treated teeth, the amount of remaining pulp tissue varied between 1 and 7.5 millimeters. After removal of the pulp, the canal was irrigated with buffered saline, a pellet of cotton soaked in 2 percent potassii iodidum iodine was inserted in the orifice

of the root canal, and the cavity was sealed with a temporary cement (type of cement was not identified). The teeth were extracted three to eight days later.

During treatment, five of the 26 teeth became significantly tender and the remainder were asymptomatic. In two cases that were markedly tender, the pulp tissue was necrotic with cellular infiltration. In the other three cases of tenderness, the pulpal stumps were well preserved from the amputation site to the apex.

No significant difference was evident between the two cutting instruments; however, the pulpal stumps appeared more frequently twisted after the use of the Antheos reamer. A total of five cases showed necrotic pulps with cellular infiltration. In four of these cases, the cut-tip Hedstrom file was used and in one case, the Antheos reamer. Loss of tissue in the central part of the pulpal stump was considered the result of tearing away of the nerves and blood vessels by the instrument and was theorized as contributing to pulpal necrosis. In 17 cases where the pulpal stump was twisted and tissue debris or dentin spicules impacted, the pulp was interpreted as well preserved from the apex to the amputation site.

This study by Nyborg and Halling was designed to compare the cutting capability of two instruments in performing partial pulpectomies with the pulp being amputated more apically than in a coronal pulpotomy, and thus does not precisely parallel the procedure in this thesis investigation. However, it showed that pulpal tissue amputated within the root canal without the application of medicaments directly on the pulp has a favorable healing potential. Even though this study was not performed under sterile conditions and the cavity

seal of a temporary cement raises a question about the potential effect of microleakage, 17 of the 26 pulpal stumps were interpreted as being well preserved. The assumption from this article and illustrated tissue sections is that the pulpal stumps in 17 cases sustained healthy conditions.

In another study of a radicular pulpotomy technique, Engstrom and Spangberg[11] used a blunted Hedstrom file to obtain an even wound surface in 23 partial pulpectomies on permanent teeth. These investigators positioned the amputation site at 0.5 millimeter to 3.5 millimeters from the apical foramen, which resulted in leaving less residual pulp tissue in the root canal than in the previous study by Nyborg and Halling.

In 1970 Mejare and associates[39] compared the use of a Hedstrom file with cut tip to an experimental instrument which resembled a smooth shank file with a pointed and sharpened curved tip for partial pulpectomy treatment. Although the surgical procedures were supposedly performed under "aseptic precautions" after the pulps were exposed, no details were given as to enamel surface disinfection, use of sterile surgical procedures, or technique used to expose the pulp canal. After partial pulpal amputation, the root canal was irrigated with physiologic saline, and a cottonwood pellet moistened with 2 percent potassii iodidum iodine was applied to the orifice of the root canal. The cavity was sealed with an unidentified temporary cement and no medicament was intentionally applied directly to the pulps. Concern for marginal leakage of the cement restoration was not mentioned. Two to five days after treatment, 10 of the 20 teeth available for histologic study exhibited twisted residual pulps, with dentin splinters having been

pushed into the pulp tissue and with evidence of a moderate to severe inflammatory infiltrate. The 10 other residual pulps had twisted tissue with dentin splinters, but the cellular infiltration was small and adequate healing was predicted for this group. No difference was found between the two types of root files in the extent of damage to the residual pulp.

In 1971 Baume and associates[40] described a radicular pulpotomy technique to maintain the vitality of the pulpal stump. An aseptic technique was stressed as mandatory. Rubber dam was used, but the procedure for disinfecting the enamel surface was not described. After direct access through the crown to the roof of the pulp chamber was obtained, the tooth surface and cavity walls received an application of formalin solution to fix the microorganisms present in this area and thereby prevent the transfer of bacteria to the amputation site. Only sterile instruments were used after this procedural step. The roof of the pulp chamber and the coronal pulp were removed with sterile, medium-sized round burs. The pulp chamber was washed with 5 percent hydrogen peroxide solution. The radicular pulp was amputated with flat-ended cylindrical reamers. Hemorrhage was controlled by irrigation with 5 percent hydrogen peroxide or calcium hydroxide applied on the end of a paper point. As the root canal was enlarged and smoothed with the flat-ended reamer, dentin chips were produced and packed in contact with the apical pulpal stump. Zinc oxide-eugenol cement was then applied as the filling material over the dentin chips.

In a subsequent publication, Baume and co-workers[41] evaluated their radicular pulpotomy procedure histologically. Six pulps from human teeth were examined 60 to 120 days postoperatively. All teeth

demonstrated progressive calcification of the pulp tissue into osteodentin at the amputation site and along the dentinal walls. Less calcification was reported where fewer dentinal chips were present. Vital tissue remained sealed off by a fibrotic barrier, under which loose connective tissue free of inflammation was present. However, some regressive changes were noted.

The technical step of amputating pulpal tissue has been performed under a wide variety of pulpotomy procedures. Armstrong and co-workers[8] used a safe-ended Number 557 fissure bur to remove the roof of the pulp chamber in monkey teeth. The coronal pulp was amputated by running a Number 4 round steel bur counterclockwise. After establishing an occlusal cavity preparation, Magnusson[16] and Schroder and Granath[9] used a round bur or a spoon excavator to amputate the coronal pulp. Anderman[42] proposed the use of electrosurgery for pulpotomies, but no histologic results were reported. In 1982 Yakushiji[43] amputated the coronal portion of the pulp with the use of electrosurgery. However, the treatment results were limited to clinical and radiological evaluations.

In a 1950 literature review Castagnola and Orlay[44] emphasized the opportunity to maintain pulpal vitality for many years with a vital amputation (pulpotomy) technique. Cleaning the tooth surfaces, applying rubber dam, and swabbing the teeth with 5 percent iodine were recommended as prerequisites. All of the following procedures were to be performed under aseptic conditions. Sharp longshank excavators and longshank rose burs were to be used for coronal pulp amputation with "care being taken not to tear it to pieces." The authors suggested applying Calxyl (calcium hydroxide with some salts of human blood

serum) to the amputated pulp and covering it with zinc oxide-eugenol followed by oxyphosphate cement and an amalgam restoration. Histologic findings of the wound area were described, including the Calxyl with dentin-splinters, tubular dentin, predentin matrix, odontoblastic layer, and normal pulp. The odontoblasts appeared normal but vacuolation sometimes occurred. The authors noted that after pulp capping and pulpotomy in patients over 30 years of age, calcific deposits, vacuolation, and reticular atrophy may be found. However, they also reported that these findings are regular features of many ageing untreated pulps.

In a 1966 pulpal study using monkeys, Masterton[45] described a pulpotomy procedure in which no dressing was applied directly to the pulpal amputation. After a rubber dam was applied, the tooth was swabbed with a quaternary ammonium compound. Box cavities with a depth of 4 millimeters were prepared in the occlusal dentin. A wide pulp wound was made in the pulpal floor and a dentin ledge was left around the pulp exposure. The coronal pulp was excised about 4 millimeters beneath the base of the box preparation with a long-shanked excavator. The amputated pulp was permitted to bleed and form a clot. A piece of platinum foil was cut to the approximate outline of the box cavity, flamed, and placed in position on the floor of the box preparation so that it rested over the opening into the root canal and did not contact the pulp tissue. The foil was covered with warm gutta-percha followed by a layer of zinc oxide-eugenol and the cavity was restored with zinc oxyphosphate cement. Histologic evaluation one month after treatment revealed that none of the 12 wounds healed. Fibrous degeneration with chronic pulpitis and abscess formation was evident. In sum,

disinfection of the tooth enamel surface was attempted but the effectiveness was not evaluated. Apparently a clean but not identified surgical technique was used. Efforts to control heat production and trauma to the pulp during cavity preparation were not cited. It appears that no direct contact was made between the platinum foil and the pulpal amputation; however, the sealing capacity of gutta-percha and zinc oxyphosphate cement to prevent marginal leakage is questionable.

In an unusual technique for performing a coronal pulpotomy on permanent molars, Britton[24] used a large fissure bur in an air turbine handpiece to open the occlusal surface as wide as possible to reveal the bleeding pulp. The cavity walls were prepared to diverge from the occlusal, and a sharp line angle was formed at the occlusal floor. All debris and carious dentin were removed. If a rubber dam was to be used, it was applied at this time and a clean surgical technique was followed using sterile instruments. A fine fissure bur was inserted into one of the exposure points, just to the level of the pulp, and three sides of the pulpal roof were joined with this cut. The cavity was carefully cleaned, so that a minimum of debris entered the pulp. An excavator was inserted into the three-sided cut and twisted until the remaining dentinal roof fractured. The roof of the pulp chamber was then removed, which left a shelf of dentin surrounding the pulp. The coronal pulp was amputated with a sharp large spoon excavator. A pledget of cotton wool was inserted in the pulp chamber to absorb the hemorrhage. Calcium hydroxide was applied to the amputated stumps of each canal. A metal plate was fitted from a matrix band to

the shelf of dentin previously established at the level of the original pulp exposure. The metal plate was flamed and secured to the shelf of dentin with zinc phosphate cement. Supposedly the metal plate was to protect the pulpal stumps from restorative procedures and to allow a space for expansion during inflammation of the injured pulp. The cavity was restored with fortified zinc oxide cement. Evaluation in eight weeks was made by removing the temporary cement and metal plate, and spraying water into the pulp chamber until all evidence of the calcium hydroxide and blood clot was removed. A bridge of hard material occluding the openings of the root canals was inspected and vitality tests against the bridge were conducted. After a positive vitality test, the bridge was covered with calcium hydroxide, fortified with zinc oxide cement, and the preparation restored with amalgam. No histologic assessments were presented.

In a 1978 essay on preserving exposed pulps Massler[46] asserted that no significant difference existed in the healing potential of medicaments applied to pulpal tissue "provided they are bland and non-irritating." He stated that it is the pulpal cells that heal and medicaments have little influence on the healing potential of the cells. Massler further stressed that a leak-proof material, such as one of the recently developed zinc oxide-eugenol compounds, should be used and covered by zinc cement or amalgam in a band or steel crown. He cautioned that leakage is the most common cause for failure in vital pulp therapy. For coronal pulpotomy, he stressed that the rubber dam must be used and the operating field disinfected, with pulpal

hemorrhage serving to remove dentinal debris, and with a clean or sterile technique being followed to obtain successful healing of the residual pulp.

A 1980 literature review of pulp therapy for primary and immature permanent teeth by Johnson and associates[47] is perhaps representative of current philosophies of pulpotomy techniques. A careful regimen that is either clean or sterile was recommended by the authors. The recommended pulpotomy procedure includes isolating the tooth with a rubber dam to avoid contamination by saliva, gaining access to the pulp through the occlusal of the crown, and excising the pulp tissue within the chamber just below the entrances to the root canals with a sterile, sharp, medium or large spoon excavator or a large sterile round bur. The pulp chamber is cleaned and hemorrhage controlled by pressing dry sterile cotton pellets over the entrances to the pulp canals. Dressings of formocresol or calcium hydroxide are applied to the pulpal amputation site. Reinforced zinc oxide-eugenol cement is placed in the pulp chamber. The tooth is restored with either a stainless steel crown or an amalgam with two applications of cavity varnish, or as an alternative a temporary restoration of reinforced zinc oxide-eugenol (IRM[a]) is placed.

In 1981 Holland and associates[48] performed pulpotomies on 40 teeth of three young dogs. The teeth were isolated with rubber dam material but no evidence was given that the enamel surfaces were disinfected or that sterile operating room procedures were followed. Using a diamond bur in a high-speed handpiece and irrigation with physiologic saline solution, access to the pulp chamber was obtained.

---

[a] The L. D. Caulk Co., Milford, DE 19963.

The coronal pulp was removed with a sterile round bur and a spoon-shaped excavator. Bleeding was controlled by washing the pulp chamber with saline and applying pressure on the pulpal stumps with sterile cotton pellets. The pulp was covered with calcium hydroxide either in distilled water or in a powder form. All cavity preparations were restored with zinc oxide-eugenol cement. Histologic evaluation 30 days after treatment showed no significant differences between the two forms of calcium hydroxide applied to the pulp. About 90 percent of the specimens demonstrated a hard tissue bridge formation in the area of the amputation site superior to a vital and noninflamed pulp.

In 1981 Ehrmann[49] presented three case histories and a literature review on the advantages of performing pulpotomies on vital immature permanent teeth using a corticosteroid as the pulpal dressing. Ledermix was recommended as the corticosteroid material because it was thought that it would not induce a calcific bridge. Calcium hydroxide was criticized for contributing to the side effects of internal resorption, diffuse pulpal calcification, or complete calcification of the canal. To prevent calcific-bridging in the pulp, zinc oxide-eugenol cement has been recommended as a dressing for pulp amputation, but this material has also been cited as producing chronic pulpal inflammation and in one reported case it resulted in complete canal obliteration. Formocresol has been widely advocated as a dressing for pulpotomy. However, formocresol-treated pulps have been recognized as producing fixation of the pulpal tissue and the pulps may develop calcific-bridging and calcific metamorphosis. Ehrmann's own pulpotomy technique includes the sterilization of all instruments and materials, application of rubber dam, amputation of the coronal pulp with a sharp

spoon excavator, and control of pulpal hemorrhage by applying cotton wool pledgets soaked in sterile saline or local anesthetic. Ledermix paste is applied to the pulp stump and covered with a layer of zinc oxide-eugenol cement and then a layer of zinc phosphate cement. The cavity is restored with composite resin or amalgam.

Ehrmann reported clinical results for 12 teeth which received this pulpotomy technique. Ten teeth were considered to have been successfully treated with continued apex formation. The apical formation for two teeth did not develop and apexification procedures were instituted. Although Ehrmann took note of the controversy over the use of topical corticosteroids on the dental pulp, he cited the anti-inflammatory effects of corticosteroids, the belief that the Ledermix used was restricted to the pulpal contact region, and the desirability of not having a dentin bridge formation to interfere with subsequent placement of a dowel pin in supporting a crown restoration.

The literature on pulp therapy techniques includes associated topics such as the dissipation of heat from tooth cutting, the thickness of coronal pulp tissue, and the importance of careful fixation of laboratory tissue specimens. Morrant[50] published a literature review on the detrimental effects to dentin and pulp tissues from the heat applied to teeth and the dehydration of dentin during operative procedures. The importance of using a coolant for cutting tooth structure was emphasized. Berk and associates[51] stated that if the coronal pulp tissue behind the pulp exposure is too thin, pulpal curettage or amputation of the entire coronal pulp is indicated. In a discussion of pulp capping and pulp amputation, Stanley[52] cited the need for immediate fixation of tissue specimens in evaluating pulpal treatment and

said it can be enhanced by cutting off the root end to permit penetration of the 10 percent formalin. Frequent changing of the 5 percent formic acid to decalcify the study tooth was recommended to prevent loss of cellular detail.

A few investigators have endeavored to secure biopsy specimens for examination of the coronal pulpal tissue. In an attempt to study the intact odontoblastic cell layer in the rat incisor, Gotjamanos[53] successfully removed the entire pulp tissue, including the peripheral odontoblastic layer. A high-speed handpiece was used to cut two longitudinal grooves in the alveolar bone surrounding the tooth. A fine straight chisel was applied to one groove with a gentle leverage and the tooth was split open to remove the pulp tissue intact.

Torneck[54] examined sections of coronal pulp tissue in four young first permanent molar teeth. Histologic sections were taken from the area of carious pulp exposure. The technique of obtaining pulpal sections for analysis included the following. Immediately after extraction, the four teeth were cracked open in a bench vise to expose the dental pulps. After initial fixation, the fractured enamel and dentin were dissected away and the coronal pulp was excised and removed. Following further fixation, the specimens of coronal pulp were prepared for study under an electron microscope. It was not possible for the investigator to orient the pulpal segments in order to identify in which direction the sections were being cut. Therefore, in evaluating the serial sections it could not be determined whether the changes noted were progressive alterations from the occlusal surface of the pulp toward the root canal.

In Magnusson's[10] pulpotomy study on 100 mandibular primary molar teeth in four-to nine-year-old children, the procedures for exicising the major portion of the coronal pulp from cariously exposed teeth were not described. However, in 40 study teeth the residual tissue over the orifice of the distal root canal was removed with an excavator. This one to two millimeters thick biopsy specimen was evaluated histologically. Analysis of the pulpal biopsy specimens revealed no direct correlation with the outcome of the pulpotomies. Magnusson stated that it would have been desirable to obtain the entire coronal pulp in the biopsy specimens, but said that would have been technically difficult. Therefore, only very small pieces of tissue adjacent to the root canal orifice were removed.

Irrigating Solutions

Various solutions have been used to irrigate following the excision of coronal pulp tissue. Several authors[12, 18, 19, 21] have reported moving directly from the pulpal amputation to the application of a hemostatic agent and/or a medicament for pulpal healing. Others have applied a wide variety of irrigating solutions before continuing with hemostasis and/or the use of a drug.

Engstrom and Spangberg[11], Andrioni and Russo[22], and others[28,48] have irrigated the pulpal amputation sites with isotonic saline. No mention was made that the saline solution was sterile. Spedding[17] recommended using any non-irritating fluid such as distilled or tap water or physiologic saline for irrigation of the amputation. He cautioned against the use of local anesthetic solution for debridement of the excision site.

Magnusson[10] and Schroder[23] irrigated the pulpal amputation site with sterile physiologic saline. In a subsequent study, Magnusson[16] cleansed the amputation site with a solution of a quaternary ammonium compound before applying formocresol.

Armstrong and associates[8] used distilled water to irrigate the cavity preparations in 40 monkey teeth and to remove debris after amputating the coronal pulp. No mention was made that the distilled water was sterile.

Edelhauser et al.[55] studied damage to corneal endothelium in rabbits and monkeys by irrigating solutions used during intraocular surgery. Balanced salt solution (sodium chloride, potassium chloride, calcium chloride, magnesium chloride hexahydrate, sodium acetate, and sodium citrate) was judged to be more effective as an irrigating solution than either 0.9 percent physiologic saline or lactated Ringer's solution, but less effective than glutathione-bicarbonate-Ringer's solution for intraocular surgery, except when glutathione and adenosine were added to the balanced salt solution.

Edelhauser and associates[56] continued to compare the toxicity of intraocular irrigating solutions on endothelium from rabbits and human donor corneas. The results confirmed that glutathione-bicarbonate-Ringer's solution followed by balanced salt solution was by far the most effective intraocular irrigating solutions for reducing endothelial cell damage and decreasing corneal thickness.

Hemostasis

Control of bleeding at the wound surface has been approached from various perspectives. Some clinicians[16, 18, 29, 57] recommend that a

treatment dressing be applied directly to the hemorrhaging wound. Others insist that a blood clot must be established before applying a treatment medication to the amputated stump.

Armstrong and associates[8] and others[17] have controlled pulpal hemorrhage with sterile cotton pellets moistened with distilled water. Some clinicians[12, 15, 21, 47, 48] control pulpal bleeding by applying dry sterile cotton pellets. A few authors[9, 14] suppress the bleeding of pulpal amputation by means of cotton pellets soaked with a saturated solution of calcium hydroxide in water.

Hansen and co-workers[19] secured hemostasis by flushing the pulp chamber with 2 percent aqueous chloramine solution and the application of sterile cotton wool pellets. Stark and associates[20] recommended placing resorbable oxycellulose against the exposed bleeding pulpal tissue. This material has been used in dentistry primarily to control hemorrhage in post-extraction sockets. The authors stated that it stopped the bleeding immediately when applied to exposed pulps.

In a study on 14 children, Kouri and co-workers[13] compared the results of formocresol pulpotomies by altering one variable. In each patient, mandibular right and left first primary molar teeth received pulpotomy treatment. In one tooth the bleeding was controlled with commercially prepared epinephrine and cotton, and for the tooth in the opposite quadrant hemostasis was obtained with sterile water and cotton. After six weeks, histologic evaluation demonstrated that the main difference between the two groups was the amount of extravasated blood present. Teeth treated with epinephrine had extravasated red cells limited to areas of the amputation site. Teeth treated with sterile water showed a diffuse red blood cell distribution in the pulp.

In a related study on hemostasis, Bahn and Mursch[58] conducted an interesting experiment on the control of bleeding from wounds created in the ears of New Zealand rabbits. The drip rate of blood flow was measured during the application of a cryoprobe, and without cold. Contrary to an accepted principle that cold at nonlethal temperatures will decrease bleeding from superficial wounds, this investigation showed that an actual increase in blood loss can occur with cold application. In vitro coagulation times and in vivo bleeding times were proportionately prolonged as the temperature decreased from 30° C to 1°C.

Marginal Leakage

Currently, dental restorations are unable to completely seal cavity preparations[59]. Leakage of microorganisms and other deleterious agents between the restoration and the cavity preparation poses a hazard to pulpal tissue at the amputation site following pulpotomy procedures.

In 1951 Armstrong and Simon[60] prepared horizontal cross sections of a small number of buccal Class V cavity preparations restored with several materials in extracted bicuspids. Autoradiographs demonstrated penetration by radiocalcium of all the materials, including amalgam (without cavity varnish), gold inlay, gold foil, oxyphosphate of zinc cement, silicate, and resin.

With the use of dyes (gentian violet or methylene blue) and glass tubes, Massler and Ostrovsky[61] in 1954 compared the sealing quality of various filling materials. They reported that amalgam (cavity varnish not used) was the most effective, with no sign of dye penetration after 180 days. Mixtures of zinc oxide and eugenol were

the next most effective in marginal sealing, with the dye penetrating only two to three millimeters along the margin after 120 days. In 1958 Weiss[62] also demonstrated, with the use of dyes, that zinc oxide-eugenol cements had much less marginal leakage than zinc phosphate cements.

A radioactive iodine solution (I^{131}) was used as a tracer by Dute and associates[63] to study the penetration around various restorative materials in Class V cavity preparations of 245 extracted teeth. The order of penetration of the iostope into the margins of the restorative materials from the greatest to the least microleakage follows: self-curing acrylics, zinc phosphate cements, silicate cements, zinc oxide-eugenol cements, silver amalgam (use of cavity varnish not reported), gold inlay, red copper cement, copper amalgam, and gold foil.

Swartz[64] refined the technique of evaluating marginal leakage of restorative materials with the use of Ca^{45}. In an *in vitro* study, cavity varnish (Copalite) aided in sealing the margins of preparations restored with amalgam, and leakage diminished with the ageing of the restoration. Although the sealing capability of a thick mix of zinc oxide-eugenol (zinc acetate crystals added) was initially effective, autoradiographs of 30-day specimens indicated considerable marginal leakage. Gilmore[65] demonstrated in an *in vivo* study that the clinical results of marginal leakage closely verified *in vitro* studies.

Going and associates[66] used crystal violet dye and radioactive sodium iodide (I^{131}) to demonstrate that all materials placed in Class V preparations were associated with some degree of marginal

leakage. Zinc oxide-eugenol cement and amalgam (without cavity varnish) showed dye penetration limited to the marginal walls, but permitted isotope penetration into the underlying dentin.

In a 1962 study Swartz and Phillips[67] placed Class I or Class V cavity preparations in extracted teeth. Autoradiographs with Ca^{45} were used to monitor isotope infiltration. The marginal leakage of amalgam restorations was greatly reduced with the use of a cavity varnish. Ca^{45} and S^{35} were used by Barber and associates[68] to show that copal resin varnish placed on the walls and floor of Class II and Class V cavity preparations which were restored with amalgam was effective in sealing margins against penetration from isotope tracers. In 1966 Dolvan[69] used Ca^{45} to compare autoradiographs of Class V cavity preparations with amalgam restorations in human teeth. The study group which had Copalite placed in the preparation and extended to the cavosurface margin presented the most effective barrier against marginal leakage, as contrasted with a control group and a group with Copalite applied only to the dentin.

An <u>in vitro</u> study on the marginal leakage of temporary sealing materials for endodontic access openings was conducted by Marosky[70]. Autoradiographs produced by the use of radioactive Ca^{45} demonstrated that Temp-Seal had the least microleakage, followed in order by Cavit, zinc oxide and eugenol cement, zinc phosphate cement, IRM, and Durelon.

Holland and associates[71] compared the influence of amalgam and zinc oxide-eugenol cement as sealing materials on the healing of inflamed pulps. Sixty pulps in dogs' teeth were exposed with a round bur to initiate pulpal inflammation. Twenty-four hours later a rubber dam was applied and a spoon excavator was used to curette pulpal tissue.

Hemorrhage was controlled by washing the cavity with saline and exerting slight pressure on the pulp with sterile cotton pellets. Twenty teeth were capped and sealed with zinc oxide-eugenol cement and 40 teeth were capped with calcium hydroxide in distilled water. Twenty of the teeth treated with calcium hydroxide had the preparations sealed with zinc oxide-eugenol cement and 20 were sealed with amalgam. No evidence was presented that a cavity varnish was used with the amalgam restoration. Histologic sections 90 days after treatment gave the following results. Of the 20 pulps capped and sealed with zinc oxide-eugenol cement, 16 had inflammation and four had necrosis. Two of the 20 pulps capped with calcium hydroxide and sealed with amalgam exhibited no inflammation, 17 were inflamed, and one developed necrosis. Of the 20 pulps capped with calcium hydroxide and sealed with zinc oxide-eugenol cement, 14 had no inflammation, 5 were inflamed, and 1 had necrosis. The results suggest that the cavity sealing material can influence the healing potential of inflamed pulps and that capping pulp tissue with calcium hydroxide and sealing with zinc oxide-eugenol cement was more effective than capping pulps with calcium hydroxide and sealing them with amalgam. The authors hypothesized that the pressure exerted during amalgam condensation could have pushed the material against the pulp tissue, producing further injury.

Wound Healing

In 1966 Masterton[72] excised coronal pulps from 46 teeth of eight monkeys. He said that a sterile field was obtained with the application of a rubber dam and topical cetrimide (Cetavlon), but no evidence was presented that the teeth were free of cultivable bacteria or that sterile operating room procedures were followed. Pulpotomies were

performed from an occlusal and incisal approach using round and fissure burs, followed by excision of the coronal pulp with sharp excavators. The wound was permitted to bleed for a few minutes and the loose clot was removed to place calcium hydroxide (Calxyl) in contact with pulpal tissue. Zinc oxyphosphate cement was used as the outer restorative material. Wound healing was observed histologically at periods ranging from one hour to three months. Masterton concluded that if the pulpal tissue was incised cleanly and hemorrhage was minimal, tubular dentin formation was induced by the calcium hydroxide dressing.

Similar pulpotomy procedures were performed on 35 human teeth over a period of 18 days to 400 weeks. Healing was the same in monkey and human specimens. The sequence of healing was as follows.

One hour after the operation: Blood-clot and debris were present on the surface of the wound. At a deeper tissue level, the superficial part of the pulp was necrotic. Farther apically, an infiltration of round cells and odontoblasts showed evidence of pyknosis. The next zone showed fibrin exudate. The remainder of the pulp was hyperemic.

Twenty-four hours after the operation: Superficially, a well-formed blood clot was attached to the underlying necrotic pulp. The necrosis had become more extensive and no sharp line of demarcation existed with the remaining pulp. Karyolysis of the cells was evident in this zone. The remainder of the pulp contained numerous dilated blood vessels, but there was only slight infiltration of round cells. The odontoblasts were considerably disturbed by the inflammation.

Three days after the operation: The clot and necrotic tissues were separated from the rest of the pulp. The pulp was hyperemic but inflammatory cells were not present in large numbers. At the site of

separation of necrotic tissue from the pulp, the connective tissue formed a dense barrier. Bundles of fibers had been laid down. The rest of the pulp was hyperemic and the odontoblasts were disturbed.

One week after the operation: The clot and necrotic tissues were separated from the rest of the pulp. The pulp was hyperemic but inflammatory cells were not present in large numbers. A dense connective tissue sheet was present on the surface of the vital pulp.

One month after the operation: The clot and necrotic layer were separated from the rest of the pulp and were disintegrating. A complete barrier composed of a calcified organized clot was present. Dentinogenesis had begun on the pulpal surface of the calcified mass, and a few tubules with cell processes passed through them. Hyperemia had almost subsided and no round-cell infiltration was present.

Isermann and Kaminski[73] reported in 1979 on a histologic study of bacterially contaminated, minimally exposed pulps in dogs. The procedure included swabbing each tooth with 70 percent ethyl alcohol, and although no rubber dam was used, the eight teeth in the study were isolated with gauze sponges. Class I cavity preparations were constructed with a sterile Number 557 bur with physiologic saline solution used as a coolant. A sterile Number 33 1/2 bur was used to closely approximate the pulp, and a small pulp exposure was created with a Number 16 endodontic explorer. The exposure site was infected with S. faecalis. The cavity was covered with gold foil and restored with amalgam. Use of a cavity varnish was not cited. Periapical lesions developed radiographically by the 15th day in all eight teeth. In another study group, 10 cavity preparations without pulp exposures were

bacterially infected. Only two of the 10 teeth demonstrated inflammatory changes and loss of odontoblastic function directly beneath the infected dentinal tubules.

Although this study did not use a sterile operating technique and the cavity seal of amalgam apparently was not ideal, since cavity varnish was not used, the response of pulpal tissue in dogs to the injurious effect of microorganisms was clearly demonstrated by the development of necrosis in all eight experimental teeth.

In a companion investigation using the same operating procedures, these investigators[74] performed direct pulp capping with calcium hydroxide (Dycal) on 10 dog teeth. The research design included the following: Two days after creating a small pulpal exposure, contaminating the pulp with S. faecalis, and restoring the tooth, they removed the restoration and capped the pulp exposure with Dycal. After 90 days, histologic evaluation revealed that nine of 10 teeth were judged as having viable pulps. Hard tissue repair was evident in five of the nine teeth. However, it is interesting that eight of the nine teeth were assessed as having some type of degenerative changes within the pulpal tissue. From this associated study, it was proposed that Dycal had some bacteriostatic effect and healing potential.

Kakehashi and associates[75] performed a classical investigation on surgical pulpal exposures. By comparing exposed pulps in germ-free and conventional laboratory rats, they demonstrated that the presence or absence of microorganisms is a major variable in pulpal healing. The coronal pulps in both groups of animals were exposed with a one-half round bur, no medicaments were applied to the pulp, no restorations were placed, and the pulp entry was left open to receive impaction of

food and debris. On the eighth day, the conventional control animals had vital pulp tissue remaining only in the apical half of the roots. Colonies of microorganisms were identified with special stains in pulpal and periapical tissues. After the eighth day, all specimens from the conventional animals demonstrated pulpal necrosis with chronic inflammatory tissue and apical abscess formation. The germ-free animals showed minimal evidence of pulpal imflammation and no devitalized pulpal tissue or apical abscess formation. Dentinal bridging was identified at 14 days and vital pulpal tissue was evident beneath the dentinal bridge. The bridging was completed by 21 to 28 days. This study demonstrated the important role that microorganisms play in adversely affecting the repair of surgically traumatized pulps which receive further irritation from food impaction.

Kalnins and Frisbie[76] studied the effect of dentin chips on the healing of exposed pulps in 71 human primary and permanent teeth scheduled for extraction in orthodontic or prosthetic treatments. Efforts were made to eliminate all dentin particles from the cavity preparation by enlarging the cavity preparation, washing out dentin particles with a water spray, permitting copious pulpal bleeding, and irrigating with a 3 percent hydrogen peroxide solution. A capping paste of calcium hydroxide and sulphathiazole was applied to the pulpal wound. Although attempts were made to prevent dentin fragments from being inserted into the pulp, 39 of the 71 teeth demonstrated dentin fragments in the pulpal wound. Another seven cases with dentin chips in the pulpal tissue from a previous study were included in this investigation, making a total of 46 cases of dentin fragments inserted in the pulpal tissue. Of these 46 pulps with dentin fragments, 29 showed

inflammation. In 18 of these 29, a diffuse pulpitis was associated with the insertion of small dentin chips at the wound site. Large dentin fragments tended to cause inflammatory reaction characterized by an accumulation of lymphocytes, plasma cells, and macrophages. Also, non-inflamed pulps with dentin fragments showed disturbances in wound healing. In both inflamed and non-inflamed pulps, the presence of dentin chips disturbed the healing. The severity of the inflammation seemed to be proportional to the total surface area of dentin chips present.

The investigators' recommendation from this study was to remove all dentin chips resulting from pulpal amputation or capping from the area of operation in order to prevent the pulp from giving a foreign body reaction which disturbs the healing process.

The significance of the effect of bacteria on pulpal wound healing was demonstrated by Armstrong and associates[8]. Although no statistical difference was evident in comparing the effects of calcium hydroxide with formocresol on pulpotomized immature monkey teeth, Brown and Brenn staining showed the bacteria were present in each tooth that had pulpal inflammation or necrosis.

Schroder and Granath[9] studied the histologic appearance of radicular pulps in eight mandibular primary molar teeth following pulpotomy. A clean surgical technique was used and sterile calcium hydroxide mixed with physiologic sodium chloride was applied to the pulpal stumps. Chronic inflammation beneath the wound surface decreased toward the apexes and in every case the pulp tissue in the

apical third was changed only slightly. Six of the 12 root canals available for evaluation demonstrated active internal resorption and 10 of these canals showed arrested internal resorption.

Magnusson[16] histologically evaluated 56 primary molars with 110 roots following the application of formocresol to coronal pulp amputation sites. Healing of pulpal tissue without inflammation was not achieved in any root canal. The application of formocresol resulted in chronic inflammation in the residual pulpal tissue of the root canals. The length of formocresol application to the pulpal amputation site had no correlation with the extent of chronic inflammation. Also, a majority of the treated roots demonstrated internal resorption.

Magnusson[10] reported poor results from pulpotomy treatments on 38 mandibular primary molar teeth using a wound dressing of calcium hydroxide. Apparently a clean surgical technique was used. Histologic evaluation revealed five teeth with evidence of healing, 31 showing inflammation and internal root resorption, and two with pulpal necrosis.

In performing 23 partial pulpectomies on permanent teeth, Engstrom and Spangberg[11] concluded from histologic examination that 16 residual pulps were successful (i.e. 11 of the 12 pulps that received a wound dressing of calcium hydroxide paste were assessed as successes and 5 of 11 pulps that received a dressing of 2 percent iodine in potassium iodide, followed in 3 to 5 days with the placement of gutta-percha and chloropercha, were evaluated as "more or less successful"). The range of histologic successful healing was based on the criterion

that the residual pulp would display no cells of exudate or, for the "more or less successful" category would display only a few cells of exudate.

Cabrini and co-workers[77] studied 54 human teeth histologically that received either a pulp cap or a coronal pulpotomy with the application of calcium hydroxide. Of the total study group, 28 cases were treated with coronal pulpotomy and seven teeth in this group demonstrated internal resorption of the dentin histologically. No pulpal inflammation was present, except that two of the seven teeth with resorption revealed small inflammatory infiltrates. The cases which received the pulp caps did not show internal resportion. The evidence of internal resorption with the pulpotomy treatment was attributed to trauma of the pulp caused by the surgical procedures or the calcium hydroxide application.

Paterson[78] compared the healing potential of exposed molar pulps in germ-free and conventional laboratory rats. Each germ-free animal received a pulp exposure, irrigation of the cavity with sterile water, drying of the cavity with paper points, application of test medicaments to the exposed pulp, and restoration of the cavity with silver amalgam in a germ-free surgery. The conventional laboratory rat received the same procedures with the apparent use of a clean surgical technique. The germ-free rat in the germ-free surgery gave excellent healing responses to the pulp capping of calcium hydroxide (Dycal), a corticosteroid (Ledermix cement), and an ethoxybenzoic acid cement. Conventional rats showed fair responses to Dycal, with several pulps showing chronic inflammation and necrosis, poor responses to Ledermix cement, and promising responses to ethoxybenzoic acid cement. The

germ-free animals which had exposed pulps left open and were maintained in a germ-free environment showed excellent pulpal response to exposure. The results of this experiment support the principle that bacterial contamination is an important factor in the response of the injured rat molar pulp.

McWalter and associates[79] histologically studied pulpal wound healing in permanent monkey teeth. The teeth were mechanically exposed, contaminated with saliva or plaque, and remained exposed for 3 to 5 1/2 hours. No aseptic procedures were used and the pulp exposure was approached from the facial surface using an air-driven high speed handpiece with a Number 701 tapered fissure carbide bur at high speed under a water coolant. An explorer point was thrust into the pink dentin to make the exposure approximately one millimeter into the pulp and one millimeter in diameter. A water spray was used to cleanse the dentin from the cavity preparation. Cotton pellets were applied to control pulpal hemorrhage at the exposure site. Keflin, Durelon, and Dycal were the three compounds under investigation as pulp-capping agents. Of the seven pulps capped with Keflin for 23 months, four showed no inflammation, one had moderate inflammation, one had severe inflammation, and one had a necrotic pulp. Of six pulps capped with Keflin for 29 months, all responded unsatisfactorily (i.e. one with moderate inflammation with partial obliteration of the coronal pulp, one with severe inflammation, and four with pulpal necrosis). Of the seven pulps capped with Durelon for 23 months and six pulps capped with Durelon for 29 months, all responded with a complete absence of inflammation. Of the seven teeth capped with Dycal for 23 months and seven teeth capped with Dycal for 29 months, none had inflammation.

All Dycal-capped pulps responded with complete reparative dentinogensis at the exposure site. Bridging was complete in only a few Durelon-capped pulps.

Berman and Massler[80] performed pulpotomies on 122 maxillary first molars of 61 albino rats. They did not explain how they maintained aseptic surgical conditions, which they said were not always possible to achieve. Also, the exact pulpotomy technique was not identified, except that two approaches were used: one from the mesial surface and the other from the occlusal surface. One-half of the pulps were covered with calcium hydroxide and half with a zinc oxide and eugenol paste. A histologic analysis was made of pulpal healing. Some of the pulps capped with zinc oxide and eugenol showed polymorphonuclear leukocytes in the superficial pulp tissue at 7 and 14 days after amputation. However, at 21 and 28 days following amputation these inflammatory cells were absent. Calcium hydroxide caused a more rapid necrosis and degeneration of the superficial layer of pulp tissue and a more rapid appearance of the primary calcific bridge. From the histologic sections, the authors concluded that unorganized blood at the amputation site appeared to retard healing while the presence of coagulated blood between the medicament and the pulp seemed to accelerate the healing process. In specimens from which the amalgam restorations were lost or when marginal leakage occurred, pulpal healing did not take place and extensive pulpal degeneration followed. These investigators proposed from their results that obtaining an adequate seal against the ingress of oral fluids overshadowed all other

considerations and that the type of medicament applied to the pulpal amputation did not appear to be the major factor in promoting bridging at the exposure site.

Schroder[57] evaluated healing after pulpotomies had been performed on 19 human mandibular bicuspids, with calcium hydroxide as the wound dressing. After 2 to 6 months, 15 of the 19 pulps showed healing histologically with a hard tissue barrier formed across the pulp. The coronal portion of the dentinal bridge had an irregular appearance with cellular and vascular inclusions and the pulpal part resembled dentin.

Sciaky and Pisanti[14] applied a paste of calcium hydroxide containing radioactive calcium to exposed pulps in dogs. Histologic sections demonstrated that the calcium in the calcium hydroxide did not enter into the formation or calcification of the new dentin bridge over the exposed pulp.

In a supposedly aseptic pulp capping study of 52 teeth in three dogs, Seltzer and Bender[15] applied various medicaments to pulp exposures and then covered the exposures with sterile asbestos fibers and amalgam. In 50 percent of the teeth, a blood clot was permitted to form before further treatment and in the remaining one-half, the treatment was applied immediately after pulp exposure. Histologic evaluation of the pulp tissue was conducted between 7 and 90 day intervals. Two pulps were treated with only physiologic saline solution before clot formation. One of these teeth demonstrated necrotic pulp and the other had calcific deposits over the pulp exposure. Three pulp exposures received only sterile asbestos fibers and an amalgam restoration after blood clot formation. Two of these pulps with no medicaments demonstrated necrotic pulps and the other one showed an intact

radicular pulp tissue with inflammatory cell infiltration. Pulp capping medicaments included calcium chloride, calcium carbonate, tricalcium phosphate, calcium hydroxide, ammonium hydroxide, alkaline phosphatase, and potassium penicillin. Calcium hydroxide was the only calcium salt which stimulated reparative dentin. Ammonium hydroxide and potassium penicillin caused pulp necrosis. The blood clot had no effect on pulp repair. In sum, all pulp exposures were placed in contact with sterile asbestos fibers. Of the five pulp exposures that received no medicaments, one had a dentin bridge with normal pulp after irrigation with normal saline and one with no medication had an inflammatory infiltrate. The remaining three of these five pulps were necrotic. The investigators theorized that the effects of the medicaments were secondary in importance to the traumatic effects of the pulp exposure technique, and that the toxic effect of the medicaments applied to the pulp appeared to hasten the necrosis of the remaining pulp tissue. Aseptic conditions cited in this study were not described and adherence to a sterile operating regimen is open to question and might be related to wound healing results.

Haruyama and co-workers[81] compared clinical and histologic assessments of wound healing using two calcium hydroxide pastes as dressings for pulpotomies in human permanent teeth. After the tooth was isolated with rubber dam or cotton rolls, the pulp was amputated with steel burs and the amputation site was cleaned with Neocleaner and dried with an absorbent material and compressed air. One of the two calcium hydroxide pastes was applied to the amputation site. Zinc phosphate cement covered the dressing and the preparation was restored with amalgam. The use of cavity varnish was not reported. It can only

be assumed that a clean pulpotomy technique was used, with no apparent effort to disinfect the enamel surface. Also, no consideration was given to dissipating the heat production from cavity preparation, or to the potential of desiccating the amputation site from the application of compressed air. Nevertheless, both groups demonstrated amazingly good results, with the pulps which received the Calvital dressing providing a superior response. Some of the pulpal pathologic changes noted in both study groups were hyperemia, bleeding, coagulation necrosis, and atrophy of the pulp. A few pulpal reactions attributed to Calxyl were round cell infiltration and suppurative inflammation.

In a related experiment, some of the same investigators[82] compared the responses of pulps amputated under conditions of isolation with rubber dam and non-isolation. The pulpotomy technique was the same as previously described by Haruyama and associates[81]. Calvital was used as the pulp dressing for both study groups. Histologic results demonstrated frequent inflammatory changes in both isolated and non-isolated conditions.

Occasionally internal resorption is found following pulpotomy procedure. Masterton[83] related this complication to unhealed wounds. He proposed two possible causes of internal resorption: (1) chronic inflammation and (2) hemorrhage. Histologic studies were conducted of unhealed pulp wounds following pulpotomy. Masterton concluded that if a hard tissue barrier was completely formed following pulpotomy, internal resorption did not occur in human or monkey teeth.

In 1898 Smith[84] published an essay on the healing capabilities of the dental pulp. He pointed out some microscopic inflammatory

responses to pulpal injury. He also alluded to the importance of treating pulp exposures with antiseptic measures to enhance the recuperative powers of the pulp.

Pereira and Stanley[85] studied the healing process of dental pulp in the dog after an exposure of approximately 0.5 millimeter and capping with calcium hydroxide. Histologic comparisons between occlusal pulp exposures and buccal pulpal penetation 120 days after treatment demonstrated equally favorable responses. The authors speculated that applying pulpal dressings from buccal pulp exposures might impair healing but this was not proven.

Bimstein and Shoshan[86] performed pulpotomies on young dogs' teeth which had incomplete apical development. An enriched collagen solution was tested as a capping agent on the assumption that collagen induces chemotactic attraction of fibroblasts. All treatments were stated to be conducted under sterile conditions; however, procedures to disinfect the enamel surface and apply rubber dam material were not mentioned. Sterile burs in a conventional-speed handpiece were used for cavity preparation and the debris was removed by air and irrigation with sterile saline solution. Hemostasis was obtained by placing sterile cotton pellets over the amputation site. The pulpotomy technique was not described. Pulp dressings of an enriched collagen solution covered with sterile dental wax or sterile dental wax only were placed in direct contact with the pulp tissue. All cavities were restored with amalgam, with no mention of cavity varnish being used. Histologic evaluation one month after pulpotomy treatment showed that all five

pulps receiving the enriched collagen solution were similar to the healthy untreated teeth. All four pulps receiving direct contact with sterile dental wax showed a diffuse inflammatory reaction.

Quigley[87] used the hamster to test the reaction of pulp tissue to capping with a calcium hydroxide-water mixture and a zinc oxide paste. After 75 days the pulps capped with zinc oxide-eugenol showed chronic inflammation and the pulps capped with calcium hydroxide demonstrated calcified bridge formation, with no odontoblastic layer and chronic inflammation persisted. From this article, only assumptions can be offered that a clean surgical technique was followed, with no attempt at disinfecting the enamel surfaces, and nothing was mentioned about the potential marginal leakage of the restoration. Yamada[88] published a brief report on healing following the resection of pulpal tissue in 101 premolar teeth in dogs. In all cases, calcium hydroxide mixed with saline solution was applied to the pulp stump. Dentin bridge formation or calcification of the remaining pulp was observed in 94.1 percent of pulp tissue amputated near the apex, in 70.5 percent of apical third resections, in 28.2 percent of middle root resections, in 47.4 percent of pulps resected at the orifice of the canal, and in 28 percent of amputations in the coronal portion of the pulp. Details of the pulpotomy technique were not given.

Hayashi[89] in 1982 examined the ultrastructure of initial calcification in wound healing following pulpotomy. Dogs' teeth were isolated with rubber dam material and the crowns disinfected with 3 percent tincture of iodine and 70 percent ethyl alcohol. Enamel and dentin were ground off with a sterile carbide bur under high speed. The pulpal amputation was performed with a sterile bur under low speed.

procedures pertaining to a clean or sterile surgical technique, irrigating solutions, or control of pulpal bleeding were not described. The amputated pulp was dressed with calcium hydroxide, covered with zinc phosphate cement, and the cavity preparation restored with composite resin. At seven days postoperatively, all eight pulps demonstrated that needle-like crystals grew among collagen fibrils. Macrophages and other large cells were found near the surface of the amputated pulp. The author concluded that events occurring during the initial mineralization of amputated pulps are similar to those found in wound healing processes that occur in other normal and pathologic calcified tissues.

Isermann and Kaminski[74] in 1979 studied histologically the pulpal response in dogs to minimally exposed and bacterially infected teeth and compared reactions between pulps capped with Dycal and pulps receiving no capping material. Eight of nine bacterially infected exposed pulps capped with Dycal showed significant amounts of vital pulpal tissue and in all eight teeth that were exposed and bacterially infected but not capped with a dressing, periapical lesions developed.

The role of leukocytes in the health of pulpal tissue was reported in a review by Stanley [90]. The leukocyte was described as the first line of defense for wound healing and phagocytosis was an essential part of the defense system. Sufficient numbers of leukoctyes must reach the site of injury as soon as possible, since they supposedly have only 30 seconds to destroy the microorganisms or they themselves will be destroyed. Several diseases, such as cyclic neutropenia, chronic granulomatous diseases, rheumatoid arthritis, sickle-cell anemia, as well as many others have an increased susceptibility to infection because of the capacity for malfunctioning phagocytosis, with

the microorganisms gaining a foothold. Healthy-appearing individuals may have other conditions that could produce phagocytic defectiveness, such as a previous splenectomy, salicylates taken in large quantities, and infections of various types. Acute alcoholism, extreme exercise, and hemodialysis have also been cited by Brubaker[91] as reducing the ability of the leukocytes to adhere to the endothelial wall during the process of margination. This lack of adherence to the endothelial lining can affect the leukoctyes' emigration into perivascular tissues to assist in phagocytosis. Stanley[90] further observed that the larger the pulp, the more vascular the tissue with which the pulp has an increased capacity to respond to injury.

In extensive literature reviews, Woehrlen[92,93] summarized the wide range of materials that have been recommended to cover exposed pulpal tissue, including weak phenol solutions, asbestos, plaster of paris, powdered ivory, vulcanized rubber, cork, oiled silk, beeswax, pulverized glass, formaldehyde, borax, zinc oxide, zinc oxide-eugenol, zinc oxide and thymol, and calcium hydroxide. Investigators had also attempted to apply other medicaments to exposed pulps such as collagen, chondroitin sulfate, sodium hyaluronate, corticosteroid-antibiotic mixture, and isobutyl cyanoacrylate. In these reviews, Woehrlen stated the commonly held philosophy of many authors that the vitality of injured pulps may be preserved if the inflammatory process can be controlled in the early stages. He pointed out that in pulpal injury, granulation tissue will form as in other parts of the body to fill in defects.

Kafrawy[94] noted that the defense cells of the pulp are histiocytes and lymphocytes, and that reparative dentinogenesis is a

defense mechanism that can function if dentinal tubules are injured. He emphasized the importance of minimizing pulp irritation by careful attention to operative procedures, the irritational potential of dental materials, microleakage of restorations and the pulpal invasion by microorganisms.

Immunology

Donlon[95] reviewed the basic principles of immunology as related to dental treatment. He stated that the reticuloendothelial system is the foundation upon which the cellular elements of the immune system function. Lymphocytes, plasma cells, and macrophages collect and interact with trapped antigens in connective tissues. Cells of the reticuloendothelial system originate from bone marrow which produces granulocytes, erythrocytes, platelets, lymphocytes, macrophages, and plasma cells. The lymph nodes, spleen, thymus, and the avian bursa of Fabricius are the essential units for the production of immune cells. Macrophages are the main phagocytic cells and they process antigens before they are recognized by lymphocytes. Lymphocytes are separated into antibody-producing B cells and T cells that are responsible for recognizing antibodies and cell-mediated immunity. Plasma cells are active secretors of antibodies. Circulating and secretory antibodies known as IgG, IgM, IgA, IgE, and IgD, along with the complement system, constitute the humoral immune response. Donlon pointed out that one of the related implications of immunology to dental treatment is immuno-suppression. This diminishing of the immune system may result in the increased occurrence of gram-negative bacterial and fungal infections,

thinning and desquamation of the oral epithelium, alteration in the oral flora, and bacterial proliferation leading to local or disseminated complications.

In considering a potential inflammatory response in an animal research model, Olsen's[96] text Immunology and Immunopathology of Domestic Animals was reviewed for information relating to this thesis investigation. Bacteria were cited as having many antigens in the cytoplasm, cell wall, capsule, flagella, and secreted toxins which are capable of eliciting individual immune responses. Macrophages were credited as normally phagocytizing and degrading greater than 90 percent of antigen. The remaining antigen is available for stimulating the lymphocytes. Also, macrophages have the responsibility of removing soluble antigen from the environment around lymphocytes, and their failure to do so paralyzes the lymphocytes.

Adamkiewicz, Pekovic, and Mascres[97] reported that the inflamed pulp contains antigens, lymphocytes, plasmocytes, IgG, IgM, IgA, and IgE antibodies, mast cells, histamine, and possibly C3. Both nonspecific and specific immunologic reactions take place in the dental pulp. The nonspecific factors kill or arrest indiscriminately the growth of most harmful microorganisms. The main nonspecific cellular factor is phagocytosis. Cells taking part in specific immune reactions are T lymphocytes and molecules of antibodies produced by B lymphocytes. Specific immunologic reactions in the pulp are provoked by antigens or haptens of five different origins. The antigens may arise from: (1) the oral tissues, (2) a systemic origin, (3) the oral flora such as dental plaque, (4) or an iatrogenic origin, such as in being released from restorative materials. In addition, exogenic

antigens are brought in with air, foods, and pharmaceuticals. Haptens are mostly systemic, iatrogenic, or exogenic. Normal pulps generally contain little antibody-producing cells and antibody molecules. As the intensity of pulpal inflammation increases, the number of cells and the amount of antibodies increase. This article implies that the health of the individual may determine the availability of antibodies to combat the antigens related to inflammation.

In a review of the relationship of immunologic concepts to endodontic diseases, Morse[98] stated that both protective and hypersensitivity reactions can occur. In addition to the serious diseases that can suppress the immune responses, physical stress, as encountered from surgery or infectious diseases, can also cause a temporary depression of immune responsiveness. Furthermore, Morse stated that immunologic reactions of either the protective or the hypersensitivity type can occur in an inflamed pulp.

METHODS AND MATERIALS

Preliminary Experiments

Before the research design for this investigation was established, a series of experiments was conducted.

Experiment Number One (Use of a Tissue-Protecting Device to Remove Dentin Over Pulpal Tissue) was an *in vitro* study using freshly extracted and chilled human third molars. Other published pulpotomy studies have not indicated that any effort was made to remove the dentin overlying the pulp without macerating the pulpal tissue. In this experiment it was desirable to remove the dentin over the buccal pulpal tissue in order to have an intact coronal pulp for the performance of a smooth incision. Furthermore, if sizable fragments of coronal pulpal tissue could be removed through the buccal preparation, the pulpal status could be examined histologically. The 25 trials carried out in Experiment Number One demonstrated that the following procedures could be performed consistently: (1) removing the remaining thin layer of buccal dentin over the coronal portion of the pulp by using a tissue-protecting device (Figure 1) attached to a high-speed handpiece without macerating pulpal tissue, (2) performing a smooth incision of the pulpal tissue with a Number 11 scalpel or an ophthalmic knife, and (3) delivering the coronal pulpal tissue in toto from the pulp chamber (Figure 2). The success of Experiment Number One had a direct relationship to the possibility of conducting the main investigation.

Experiment Number Two (*In Vivo* Trials for New Pulpotomy Procedures) was actually a pilot study for the main investigation. Since the tissue-protecting device performed favorably on extracted human teeth, the design of Experiment Number Two tested the performance of a new pulpotomy technique on three first permanent molar teeth of a dog, using sterile operating room procedures. After the dog was anesthetized by intravenous injection of sodium pentobarbital and all gingival tissue and teeth were washed with Betadine Surgical Scrub[a] for three minutes, clinically healthy study teeth received the following antimicrobial regimen. A 10 percent hydrogen peroxide solution was applied followed by a 2 percent tincture of iodine solution. After a sterile rubber dam and frame were secured around the study tooth, a 30 percent hydrogen peroxide solution was applied followed by a 2 percent tincture of iodine solution. Each solution maintained the enamel surface moist for approximately 60 seconds. A culture was secured from the buccal surface of the study tooth and inoculated in thioglycollate medium. Using a high-speed handpiece and Number 330 bur, a Class V preparation was prepared on the buccal surface. Sterile water was applied to the study tooth to maintain the operating site free of debris and to dissipate heat by the dental drill. A second preparation was completed deeper within the dentin but leaving a shoulder of at least 0.5 millimeter around the periphery of the initial cavity outline. The special tissue-protecting device permitted removal of the buccal dentin over the coronal pulp without gross mutilation of tissue. The coronal portion of the pulp was excised with an ophthalmic knife, removed through the buccal cavity preparation in fragments, and dropped into

---

[a] Purdue Frederick Company, Norwalk, CT

formalin. The amputation site was irrigated with a physiologic balanced salt solution[a]. Hemorrhage was controlled by applying sterile cotton pledgets to the amputation site. A blood clot was established.

A sterile plate of 0.006 inch stainless steel was tailored and adapted to the ledge of the Class V preparation to serve as a diaphragm between the space occlusal to the pulpal amputation site and the cavity floor. The stainless steel diaphragm was cemented by using a thin mix of carboxylate cement[b], and a premixed temporary filling material[c] of zinc oxide was applied to the cavity preparation and tapped to place with cotton pledgets.

At 36 days following treatment, the maxillary left first permanent molar was extracted in toto, and at 21 days following treatment, the maxillary right first permanent molar and the mandibular right first permanent molar were also extracted in toto, processed for histologic preparation, and stained with hematoxylin eosin.

Cultures of the enamel surface taken following the final application of antimicrobial solutions for each study tooth showed no cultivable bacteria.

The pulpal biopsies demonstrated loose fibrous connective tissue and Brown and Brenn-stained sections revealed no bacteria. Histologic sections of the pulpal tissue in the root canals revealed a heavy

---

[a] Balanced Salt Solution, Alcon Laboratories, Incorporated, Fort Worth, TX

[b] Durelon, Premier Dental Products Company, Norristown, PA

[c] Cavit-G, Premier Dental Products Company, Norristown, PA

inflammatory infiltrate at the amputation site (Figures 3 and 4) and an oblique band of fibrous connective tissue was formed in an attempt to wall off the heavy inflammatory infiltrate. A mild inflammatory response formed beneath the fibrous band of tissue. Brown and Brenn-stained sections revealed suggestive evidence of a small number of bacteria in the root canals. It was theorized that the invasion of inflammatory cells resulted from the introduction of bacteria at the operation site or from leakage of the cavity seal by the temporary filling material (Cavit-G), rather than as a response to the surgical trauma of the pulpal tissue.

Experiment Number Three (In Vivo Procedures for Rendering the Tooth Surface Free of Cultivable Bacteria) was designed to investigate the correlation between the increments of time used to maintain enamel surfaces moistened with test solutions and the resultant positive or negative cultures for the presence of bacteria.

Six dogs (A, B, C, D, E, and F) were anesthetized with sodium pentobarbital and all procedures in this experiment were performed under sterile operating room conditions with the exception of controlling the quality of room air.

The treatment of enamel surfaces began with three minutes of generalized scrubbing of all easily accessible enamel surfaces with Betadine solution. This washing was followed by the application of 10 percent hydrogen peroxide and 2 percent tincture of iodine, which in turn was followed by rubber dam isolation of the study teeth, and then by the application of 30 percent hydrogen peroxide and 2 percent tincture of iodine. For a given tooth, each of the four solutions used

remained on the enamel surface for the same number of seconds. The precise time interval for application of each solution was carefully measured by an assistant using a stop watch.

Forty-five cultures were obtained at various periods during the study. They were obtained by rubbing a sterile cotton applicator moistened with sterile water over the facial surface of the designated tooth, and placing it in a random numbered tube of thioglycollate medium[a]. The tubes of inoculated medium were incubated at 37°C and evaluated daily for five days, using a coded numbering system for each tube. The absence of viable cells in negative cultures was substantiated by gram-stains and subculturing.

As shown in Table I, Group I (the controls) consisted of nine premolar teeth and one molar tooth, including five which received no treatment and five in which the enamel surface received only a topical rubbing with Betadine solution during a three-minute scrub of all erupted teeth.

Group II consisted of a total of 11 premolar, molar, and cuspid teeth in which the sequential order of applying the antimicrobial solutions and rubber dam was carried out for periods of 5, 10, or 15 seconds.

Group III included a total of 24 premolars and molars in which the sequential order of applying the antimicrobial solutions and rubber dam was carried out for periods of 30, 60, 90, and 120 seconds.

---

[a] Difco Laboratories, Detroit, MI

All 10 cultures for Group I, the controls, demonstrated a positive result for bacteria. Five teeth of three dogs in this group had received no treatment. An untreated tooth in Dog C demonstrated streptococci, gram-negative rods and gram-positive rods; and two untreated teeth in Dog D contained streptococci and a gram-negative rod having characteristics similar to Proteus. Five teeth of three dogs were cultured immediately after all teeth had received only the generalized, three-minute scrub of Betadine solution to all easily accessible enamel surfaces. All five cultures were positive. One sample demonstrated streptococci and gram-negative rods. Cultures from two Betadine-scrubbed teeth of another animal contained streptococci and a gram-negative rod having characteristics similar to Pseudomonas.

Of the 11 teeth in Group II, 10 cultures were negative. This group included trials for solutions that had each been applied for 5, 10, or 15 seconds. Chi Square analysis demonstrated that the time periods used in this group were effective in producing an enamel surface free of cultivable bacteria at the 0.001 level. X^2 was 17.35, which was significant beyond the 0.001 level. (At 1 df, $X^2_{0.001} = 10.83$.)

Group III consisted of 24 teeth which had received the previously outlined regimen of antimicrobial solutions for the following periods: 30, 60, 90, and 120 seconds. In this group only one culture was positive. Using the Chi Square analysis, the method used for obtaining an enamel surface free of cultivable bacteria was effective at the 0.001 level. X^2 was 29.58, which was significant beyond the 0.001 level. (At 1 df, $X^2_{0.001} = 10.83$.)

When the data from the three groups were compared, the Chi Square analysis was 35.39, which was significant beyond the 0.001 level. (At

2 df, $X^2_{0.001}$ = 13.82.) These results confirmed that a significant difference existed between the three groups. Thus, individual Chi Square analysis was performed with each possible combination of group pairs.

The Chi Square analysis between Groups I and II yielded a Chi Square of 17.35 which was significant beyond the 0.001 level. (At 1 df, $X^2_{0.001}$ = 10.83.)

In comparing Groups I and III, the Chi Square analysis revealed a significant difference. X^2 was 29.58, which was significant beyond the 0.001 level. (At 1 df, $X^2_{0.001}$ = 10.83.)

In a comparison of Groups II and III, the Chi Square analysis showed no significant differnce. X^2 was 0.339, which was not significant at the 0.05 level. (At 1 df, $X^2_{0.05}$ = 3.84.)

In sum, the statistical analysis revealed a significant difference between the positive and negative cultures in Group II and in Group III. Furthermore, a significant difference was documented between Group I (control) and the experimental trials (Group II and Group III). Enamel surfaces in dogs were rendered free of cultivable bacteria whether the application of four solutions and the rubber dam, with maintenance of a moist surface for each solution, lasted only five seconds or for periods up to 120 seconds of contact time for each solution. It seems reasonable to conclude that the data support the use of the experimental antimicrobial regimen employed in this investigation for freeing the enamel surfaces of dogs' teeth of cultivable bacteria, if entry into the pulp chamber is anticipated.

As previously mentioned, the inflammatory infiltrate present in the pilot study (Experiment Number Two: <u>In Vivo</u> Trials for New Pulpotomy

Procedures) was judged to be related to the introduction of bacteria at the site of operation or subsequently at the cavity seal. Therefore, two experiments were conducted to study the possiblility of reducing the invasion of microorganisms at the pulpal amputation site.

Experiment Number Four (The Effect of Handpiece Operation on Growth of Cultivable Bacteria) was performed to verify that a surgical handpiece should be used in sterile surgery in place of a conventional high-speed handpiece.

A high-speed handpiece and two surgical handpieces were sterilized with ethylene oxide gas. The tissue-protecting devices and corresponding tailored burs were sterilized with freshly prepared, 2.0 percent, glutaraldehyde (Cidex-7). Using a 10-minute surgical scrub, sterile operating room procedures were followed in the animal surgery. The high-speed handpiece with a sterile Number 35 bur was operated from the central system of compressed air at 200,000 to 250,000 revolutions per minute. The surgical handpieces with Number 69L burs operated from an H tank of nitrogen in the range of 60,000 to 90,000 revolutions per minute.

The handpieces adapted with their corresponding tissue-protecting devices and burs were individually held approximately six inches from a hand-held Petri dish containing trypticase soy agar supplemented with 5 percent whole sheep blood. All three handpieces were continuously run for their individual trials at variable speeds for 30 seconds. Two trials were completed for each of the three handpieces, and the six Petri dishes were incubated at 37°C for a period of four days.

The operation of surgical handpieces did not produce cultivable bacteria at the operation site, whereas the conventional high-speed

handpiece demonstrated a positive culture of beta-hemylolytic gram-positive coccus similar to Staphylococcus. Although considerable handpiece speed had to be sacrificed because surgical handpieces were used, removing the capability of a handpiece to introduce bacteria at the site of operation was an important factor in the design of the main study.

Cavit-G was selected for restoring Class V preparations in the pilot study (Experiment Number Two) based on the work by Marosky[70]. In efforts to prevent the introduction of microorganisms into the amputation site through leakage at the cavity seal, Experiment Number Five (In Vitro Leakage Trials for Materials) was designed to compare the leakage capabilities of four materials by the use of autoradiographs.

Class V cavity preparations were constructed with a Number 56 carbide bur using a high-speed handpiece in extracted human molar teeth that were kept moistened in water since removal. The preparation was formed in the cervical third of the buccal surface. The outline form extended mesial-distally, the full extent of the buccal surface, and was approximately three to four millimeters occlusal-cervically. The cavity floor was approximately three millimeters in depth from the cavosurface. After cavity preparation, each tooth in this study was dried with air, and the cavity preparation was wiped with a cotton pledget containing isopropyl alcohol, and again dried with air. S. S. White MQ Lubricant was then applied to the cavity preparation with a

cotton pledget. Using the brush-on technique, an unfilled resin[a] was used to construct a thin resin diaphragm of approximately one millimeter in depth and a plastic pin[b] was placed in the center of the diaphragm (Figure 5).

After polymerization, the resin diaphragm was withdrawn, immersed in isopropyl alcohol, and thoroughly dried. The cavity preparation was washed with a cotton pledget of isopropyl alcohol, and the cavosurface margins in the enamel were freshened with the use of a Number 56 carbide bur in a high-speed handpiece. The cavity preparation was again washed with isopropyl alcohol and thoroughly dried.

The preparation received a generous coating of a solution[c] to dry, clean, and remove oil from the preparation and cavosurface margin, and the resin diaphragm was positioned in the Class V preparation.

Nine teeth were used in these preliminary trials. Three teeth were restored with an intermediate restorative material[d] (two drops of liquid to two level scoops of powder), two teeth with amalgam[e] (Dispersalloy-ratio 1:1) and two coats of a cavity varnish[f], two teeth with a premixed temporary filling material[g] (Cavit-G), and two

---

[a] Sevriton, L.D. Caulk Company, Milford, DE

[b] Williams Gold Refining Company, Buffalo, NY

[c] Cavilax, Premier Dental Products Company, Norristown, PA

[d] IRM, L.D. Caulk Company, Milford, DE

[e] Dispersalloy, Johnson and Johnson Dental Products Company, East Windsor, NJ

[f] Copalite, Cooley and Cooley, Limited, Houston, TX

[g] Cavit-G, Premier Dental Products Company, Norristown, PA

teeth with a polycarboxylate cement[a] (Durelon, two drops of liquid and one portion of powder). The study materials were applied to the respective cavity preparations. The teeth were then immersed in a water bath for a three-week period.

Autoradiographs of the nine study teeth were prepared by Swartz[b] to demonstrate the microleakage trends. The scale for grading autoradiographs developed by Marosky[70] was used to judge the results. No leakage was assigned to the material if the isotope did not penetrate the cavosurface margin. If the autoradiograph demonstrated a slight amount of leakage between the restorative material and the tooth margin, the material was considered to have slight marginal penetration. If the marginal penetration extended to the base of the restoration (i.e. to the resin diaphragm) the leakage pattern was graded as moderate. If the isotope penetrated completely around the restorative material, the leakage was considered severe.

Intermediate Restorative Material: One restoration dropped out of the preparation while in the water bath; therefore, it was considered as having severe leakage capability and was not evaluated by autoradiograph. Two restorations demonstrated leakage of the radioisotope for the full extent of the occlusal and cervical walls of the intermediate restorative material restoration (Figure 6.1) and were judged as having a moderate penetration.

---

[a] Durelon, Premier Dental Products Company, Norristown, PA

[b] Swartz, M.L.: Professor of Dental Materials, Indiana University School of Dentistry.

Dispersalloy with cavity varnish: The two restorations demonstrated a slight leakage of the radioisotope (approximately 0.5 millimeter) which was present at the occlusal and cervical cavosurface margins (Figure 6.2).

Cavit-G: Two restorations demonstrated penetration of the radioisotope for the full extent of the occlusal and cervical cavity walls, as well as the buccal wall next to the resin diaphragm. Cavit-G was graded as having a severe marginal leakage pattern. Furthermore, these two restorations demonstrated that the isotope penetrated the complete Cavit-G material (Figure 6.3).

Durelon: One of the restorations dropped out of the preparation while in the water bath; therefore, it was considered as having severe leakage capability and was not evaluated by autoradiograph. The other Durelon restoration was retained and demonstrated leakage of the isotope for the full extent of the occlusal, cervical, and buccal cavity walls (Figure 6.4), and was assessed as permitting severe leakage.

The trials in this experiment compare reasonably well with the work of Marosky in regard to IRM and Durelon, in that both studies showed that these two materials were capable of severe marginal leakage. However, these trials ranked Cavit-G with a capability of severe marginal leakage that was not evident in the Cavit material used by Marosky.

At the time of these trials, Temp-Seal and Cavit, used by Marosky[70] with best results for preventing leakage, were not available. Temp-Seal had been withdrawn from the market under the direction of the Federal Drug Administration. Cavit-G replaced the product Cavit on the market and was essentially the same material.

A very large Class V preparation was used in these trials and in the pilot study, and an extensive cavity preparation was planned for the proposed main investigation. The extensiveness of the preparation (i.e. averaging 4 millimeters X 10 millimeters) required considerably more material to restore the preparation than was used in the lingual access opening of Marosky's study. Therefore, the opportunities for shrinkage of the material would be greater in the extensive cavity preparation than in the small lingual opening into the root canal, increasing the opportunity for leakage. The capability of Cavit-G to permit leakage throughout the mass of material in addition to leakage at the cavosurface margin was an unexpected finding.

The results of these trials have important implications for pursuing another study with an expanded sample size to confirm the trends of these trials, as well as having a potential direct application for other procedures in pedodontics, such as reinforcing the sedative dressing during a convalescent period in the use of indirect pulp therapy.

In sum, amalgam with two coats of cavity varnish proved to have the least leakage capability as compared with IRM, Durelon, and Cavit-G.

Main Study

Five dogs of mixed breeds were used in this investigation, which was conducted at the Wishard Memorial Hospital Animal Surgery. Each of the animals had received an extensive abdominal incision (for a medical research project) approximately 14 days before this study began. However, the medical staff of the animal quarters judged each of the five

dogs to be in a healthy condition. In each of the five animals (Dogs A, B, C, D, and E), the maxillary left permanent premolar$_4$ was used as the study tooth.

A tissue-protecting device and tailored 69L bur were sterilized by a 17-hour immersion in freshly prepared 2.0 percent glutaraldehyde[a] solution and rinsed in sterile water. All other instruments and supplies that were not packaged in a sterile condition by the manufacturer were sterilized with ethylene oxide gas and aerated.

Anesthesia was obtained by intravenous injection of sodium pentobarbital[b] in a posterior limb using a dose of 1 cc. per 5 pounds of body weight. The syringe was anchored throughout the procedure so that anesthesia could be prolonged as necessary. All gingival tissue and teeth of the study animals were washed with Betadine Surgical Scrub[c] for approximately three minutes. The foam resulting from the Betadine scrub was removed by using sterile gauze sponges.

The operating room team for this study received a comprehensive review and practice session about sterile operating room preparations, surgical scrub procedures, and the maintenance of sterile techniques from a surgical nurse in obstetrics at University Hospital.

After donning surgical hats, masks, and shoe covers, the investigator and sterile nurse performed a 10-minute hand and arm surgical scrub with Betadine Surgical Scrub, gowned and gloved. Sterile operating room procedures were conducted throughout the five operations.

---

[a] Cidex-7, Johnson and Johnson Dental Products, Skillman, NJ

[b] Nembutal, Abbott Laboratories, North Chicago, IL

[c] Purdue Frederick Company, Norwalk, CT

A sterile pediatric laparotomy drape was applied to cover the dog, with only the head exposed through the aperture in the drape.

Using sterile tongue blades, a Mott mouth prop was inserted in the right side of the oral cavity. The clinically healthy study tooth for each animal received the following antimicrobial treatment: A solution of 10 percent hydrogen peroxide was applied with a sterile cotton applicator for 30 seconds. A 2 percent tincture of iodine solution was applied with a sterile cotton applicator for 30 seconds. A sterile plastic surgical drape[a] with a 2 1/2-inch aperture was adapted to the study tooth. A sterile rubber dam and frame were secured around the study tooth with a Number 200 clamp. A 30 percent hydrogen peroxide solution was then applied to the study tooth with a sterile cotton applicator for 30 seconds, followed by the application of a 2 percent tincture of iodine solution for 30 seconds. The precise time period for application of each solution was carefully measured by an assistant using a stop watch. The buccal surface of the study tooth was wiped dry with a sterile cotton applicator. A cotton applicator moistened with sterile water was wiped across the buccal surface of the study tooth and inserted in a tube of thioglycollate medium. The culture was incubated at 37°C for a five-day period. In addition, cultures were taken and incubated in thioglycollate medium for each study tooth after the following procedures: initial preparation, diaphragm adaptation, completion of second preparation, pulpal excision and blood clot, and diaphragm cemented.

---

[a] Steri-Drape, Surgical Products Division, 3M Company, St. Paul, MN

Two surgical handpieces[a] (estimated 60,000-90,000 r.p.m.) and hoses, which were sterilized in ethylene oxide, were connected to an H tank of nitrogen using a sterile technique to prevent contamination of the handpieces. The valve on the H tank was gauged to deliver 130 pounds per square inch of nitrogen to the handpiece.

After the antimicrobial agents were applied to the enamel surface of the study tooth and a bacterial culture was obtained, an operating microscope, properly covered with a sterile plastic drape, was used throughout the procedures until the amalgam was inserted in the cavity preparation.

A Class V preparation was prepared on the buccal surface of each study tooth using a sterile Number 57L carbide bur. The cavity floor extended approximately one millimeter into the dentin. To maintain the operating site free of debris and to dissipate heat produced by the dental drill, sterile water was applied to the study tooth with a sterile 50 cc. syringe whenever the drill was used.

Prior to the day of operation, dogs' dried skull specimens which contained teeth were used to design several potential sizes of stainless steel wire mesh diaphragms. This fabrication included welding an orthodontic bracket to each diaphragm to serve as a handle, polymerizing a thin central portion of Nuvafil[b] (Figure 7), labeling, and sterilizing with ethylene oxide. In the operating room, a diaphragm was constructed by selecting one of the sterile, partially preformed surgical stainless steel wire screens of appropriate size. The wire

---

[a] Ace Hall Type Drill, Brockton, MA

[b] L.D. Caulk Company, Milford, DE

screen was adapted to the buccal wall of the initial preparation. A film of filled resin[a] was applied to the wire screen and polymerized with ultraviolet light[a].

The polymerized diaphragm was removed and undercuts established at the dentinal-enamel junction in the four corners of the preparation with a sterile Number 35 carbide bur. Following completion of the initial preparation, a sterile Number 4 carbide bur was used to prepare a similar preparation deeper within the dentin but leaving a shoulder of at least 0.5 millimeter around the periphery of the initial cavity outline.

Following a one millimeter exposure of the pulpal tissue, a special tissue-protecting device (Figure 8) was used to remove all the buccal dentin of the second (inner) cavity preparation, thereby exposing the pulpal tissue for excision. For Dogs A, B, D, and E, the coronal portion of the pulp was excised with a 59M Beaver eye knife[b]. The amputated coronal portion of the pulp was delivered from the pulp chamber through the buccal cavity preparation in fragments with a sterile cryoextractor[c] (Figure 9) which is used in ophthalmic surgery to remove cataracts. The pulpal biopsy tissue was dropped into a bottle of 10 percent formalin solution. The coronal pulp of the study tooth in Dog C was amputated with a Number 4 carbide bur and the macerated fragments irrigated from the pulp chamber. Following pulpal excision, the amputation site was irrigated with a physiologic balanced salt

---

[a] L.D. Caulk Company, Milford, DE

[b] Rudolph Beaver, Incorporated, Belmont, MA

[c] Alcon Laboratories, Incorporated, Fort Worth, TX

solution[a] isotonic to the tissues of the eye (sodium chloride, potassium chloride, calcium chloride, magnesium chloride hexahyrate, sodium acetate, sodium citrate, and water). Hemorrhage was controlled by applying sterile cotton pledgets to the amputation site. A blood clot was established.

The previously constructed diaphragm was adapted to the dentinal ledge of the Class V preparation to serve as a barrier between the space occlusal to the pulpal amputation site and the cavity floor. The diaphragm was cemented with a thin mix of carboxylate cement[b] (three drops of liquid to one measure of powder) and a very fine restorative brush. After the tacky stage of the cement was reached, two coats of cavity varnish[c] were applied to the cavity preparation with a cotton pledget. The cavity was restored with a premeasured alloy-mercury mixture[d] (ratio 1:1).

Following the pulpotomy procedure, the study tooth in each dog was scheduled for extraction in toto, 21 days later. In addition, a maxillary right permanent premolar$_4$ was planned for extraction to serve as a control specimen. The apex of each root was cut off with a sterile Number 557 bur in a high-speed dental drill at approximately the apical third of each root, and the tooth was dropped into 10 percent formalin solution. The tooth was then decalcified in five percent formic acid,

---

[a] Balanced Salt Solution, Alcon Laboratories, Incorporated, Fort Worth, TX

[b] Durelon, Premier Dental Products Company, Norristown, PA

[c] Copalite, Cooley and Cooley, Limited, Houston, TX

[d] Dispersalloy, Johnson and Johnson Dental Products Company, East Windsor, NJ

imbedded in paraffin, sectioned in a buccolingual plane at thicknesses of approximately seven microns, and stained with hematoxylin eosin. Approximately every third slide was evaluated with light microscopy.

Several selected histologic sections of pulpal tissue remaining in the areas of the pulp chamber and root canals, as well as pulpal biopsies, were processed with Brown and Brenn-stain for evaluation with light microscopy.

RESULTS

Microbiologic Findings

Cultures of the tooth surfaces immediately following the antimicrobial treatment resulted in all negative cultures, except in Dog A which was inadvertently not tested. After the initial preparation, Dogs A and B showed positive cultures, but the study teeth in the other three animals were free of cultivable bacteria. Following the adaptation of the diaphragm to the initial cavity preparation, only the preparation in the study tooth for Dog A showed a positive culture. Cultures taken upon completion of the second (inner) preparation resulted in positive cultures for Dogs A, B, and E. After pulpal amputation and blood clot formation, only Dog B demonstrated positive evidence of bacteria. After the diaphragm was cemented, all study teeth showed negative cultures. Table II summarizes the results of efforts to keep the tooth surface free from cultivable bacteria after each of the five major procedures.

Since Dogs A and B had positive cultures, whereas Dogs C, D, and E had negative cultures (except one positive result for Dog E), Dogs A and B were placed in Group I and Dogs C, D, and E were assigned to Group II. The rationale for separating the study sample was based on the post-study observation that as the study progressed from Dog A through E, the operating team became more proficient in applying the Steri-Drape as an integral part of the antimicrobial regimen in protecting against contamination from the animal's facial hair and whiskers.

In a comparison of Group I (Dogs A and B) and Group II (Dogs C, D, and E), the Chi Square analysis showed a significant difference: X^2 was 7.95, which was significant beyond the 0.01 level. (At 1 df, $X^2_{0.01} = 6.64$.) Since the sample size was limilted to five animals, the Yates correction for small cell numbers was used and reduced X^2 to 5.66, which was significant beyond the 0.05 level. (At 1 df, $X^2_{0.05} = 3.84$.)

The first three treatment procedures were compared to the last three procedures for each dog to determine if a significant difference existed in producing a positive or negative culture as progress was made through the six major steps of the operation. In this analysis, $X^2 = 0.18$ which was not significant for obtaining a positive culture as the different surgical procedures were used.

Comparing the findings on the chance that 50 percent of the cultures would be positive and 50 percent would be negative, the anlaysis revealed that $X^2 = 8.54$, which was significant beyond the 0.01 level. (At 1 df, $X^2_{0.01} = 6.64$.) Therefore, the chance was less than one in 100 of finding 22 negatives of 29 cultures (Table II).

When the culture results were compared in relation to the individual procedures to determine if a particular step in the entire operation significantly contributed to a positive or negative culture, there were no significant differences. Between dogs there were no significant differences except between Dog A (with three positive cultures) and Dogs C and D (with no positive cultures) which yielded a X^2 of 4.28, which was significant beyond the 0.05 level. (At 1 df, $X^2_{0.05} = 3.84$.) However, applying the Yates correction for small cell numbers, X^2 was 1.92, which was not significant.

Pulpotomy Procedures

In each of the five experimental pulpotomy operations for Dogs A, B, C, D, and E, the buccal dentin overlying the coronal pulp was effectively removed without grossly disturbing the morphologic outline of pulpal tissue. In Dogs A, B, D, and E, an ophthalmic knife performed adequately in severing the coronal portion of the pulp for its removal through a buccal preparation. However, no coronal pulpal tissue was able to be delivered as a complete segment. In Dog C, a Number 4 carbide bur was used for a macerating type of pulpal amputation.

Following pulpal amputation in all five experimental operations, hemorrhagic exudate successfully clotted without the application of medicaments and a diaphragm was effectively secured without touching the pulpal amputation site, as a shield between the pulpal tissue in the root canal and the restorative material.

Histologic Analysis of Radicular Pulp

Histologic evaluation of the pulpal tissue for the maxillary left permanent premolars$_4$ for Dog A (Figure 10 -- limited to a 14-day specimen, because the Animal Surgery required terminating the use of the dog), Dog B (21-day specimen), and Dog C (21-day specimen) demonstrated coagulation necrosis. Very loose collagen fibers and a sparse presence of fibroblasts were observed. Inflammatory cells were not present in the pulpal tissue. A disrupted odontoblastic layer was evident with no odontoblasts.

No histologic tissue sections were obtained from Dog D, because the animal quarters (Wishard Hospital) mistakenly destroyed this animal 15 days after the pulpotomy was performed.

The pulpal tissue for the maxillary left permanent premolar$_4$ for Dog E (21-day specimen) was evaluated histologically. Figure 11 demonstrates a mild inflammatory infiltrate at the amputation site (A). Lymphocytes and polymorphonuclear neutrophils were identified as the primary inflammatory cells. An odontoblastic layer was present throughout the tissue section; however, it was markedly disrupted in the area of pulpal amputation. The predentin layer was missing in the area adjacent to the amputation site. Beneath the mild inflammatory area at the amputation site, the pulpal tissue, as shown in Figure 11 (B), demonstrated abnormally loose connective tissue characteristic of pulpal edema. Also, in this area a few examples were evident of the dentinal wall showing a scalloping pattern representative of internal resorption. Moving farther apically, as in Figure 11 (C), the tissue resembles normal pulpal tissue.

Figure 12 shows two high-power magnifications of the amputation site. The mild inflammatory infiltrate can be identified, as well as capillaries filled with erythrocytes. Very loose connective tissue and fibroblasts are also evident.

The maxillary right permanent premolar$_4$ for Dog E received no treatment, and it was extracted to serve as a control. Histologic evaluation of this tooth (Figure 13) demonstrated normal-appearing pulpal connective tissue and odontoblastic layer. Some vacuolation of the odontoblastic layer was evident and possibly resulted from inadequate fixation.

Brown and Brenn-stained sections of selected slides gave the following results. The pulpal tissue in the root canals of Dog A demonstrated only suggestive evidence of a very small number of

bacteria in the distal root section, and the mesial root section revealed only a few widely scattered bacteria. The pulp chamber section (area of pulpal amputation) was free of bacteria. For Dog B, the pulpal tissue in the distal root gave no evidence of bacteria and the mesial root revealed only a few bacteria. The pulp chamber section showed a small number of bacteria in clumps of debris on the cervical dentinal ledge of the preparation characteristic of gram-positive cocci. Dogs C and E presented no evidence of bacteria in the pulpal tissues of the root canals or in the pulp chambers (pulpal amputation sites).

Pulpal Biopsies

In each of the five dogs (A, B, C, D, and E), after the buccal-dentinal wall was removed with the tissue-protecting device, the coronal pulp was excised. A 59M Beaver eye knife was used for the amputations in Dogs A, B, D, and E. In Dog C, the pulp was excised with a Number 4 carbide bur. In each animal, the coronal portion of the pulp was delivered in fragments (usually in two or three sections). The morphology of the coronal pulp could not be maintained. In Dogs A, D, and E, the excised pulpal tissue was delivered from the coronal portion of the tooth crown with the aid of a microphake (Figure 9). For Dog B, a cryophake (Figure 9) was used to remove the pulpal biopsy and for Dog C, no pulpal biopsy was obtained because the pulp was amputated with a bur.

Histologic sections of the pulpal biopsies were prepared for Dogs A, B, and E. Evaluation of the pulpal biopsies demonstrated the following: loose fibrous connective tissue, fibroblasts, disarranged odontoblasts, dentin chips, capillaries, and erythrocytes.

Figure 14 illustrates a portion of a large section of the pulpal biopsy for Dog E. Normal-appearing pulpal tissue was present with deposits of fibrin at the periphery of the specimen and disarranged odontoblasts. A higher magnification of the central portion of the biopsy for Dog E is shown in Figure 15.

Brown and Brenn-stained sections of the pulpal biopsies for Dogs A, B, and E revealed no evidence of bacteria.

Blood Values

In designing this study, the use of the five dogs was arranged with permission of the medical researchers at Wishard Memorial Hospital Animal Surgery, who indicated that each dog would be available for this study approximately two weeks following their own project, and that the animals would be in good health. Each of the five dogs had received an abdominal incision approximately two weeks prior to initiating the dental operating procedures.

Postoperatively, it was discovered that Dogs A, B, C, and D were given an autotransfusion lasting approximately two hours each. It was further learned that Dog E did not receive an autotransfusion, but received an abdominal incision as an operated control, one liter of Ringer's lactate, and blood from a donor dog.

The medical research team of the Wishard Memorial Hospital Animal Surgery apparently concluded that if the dogs were still alive after the autotransfusion procedures and did not present overt signs of ill health, they were judged as healthy animals. Several months after the histologic sections were prepared and analyzed, the investigator inquired from the medical researchers about records of the dogs'

experiences with the medical research project. The medical data listed below were provided. The data for Dogs B and D were missing. The figures for Dog C were particularly sketchy since many of the values were rated as "clotted". The values for Dog A (a typically autotransfused dog) compared to Dog E (the control animal) were:

White blood count:

Dog A had a baseline value of 11,680 with a 24-hour value of 20,300 and a one-week reading of 29,000.

Dog E had a baseline value of 4,500 with a 24-hour value of 36,000 and a one-week reading of 18,500.

Hemoglobin:

Dog A had a baseline hemoglobin of 14.5 with a 24-hour value of 8.5 and a one-week value of 10.7.

Dog E had a baseline hemoglobin of 14.6 with a 24-hour reading of 16.3 and a one-week value of 10.9.

The animals in this study had a disruption in their immune system. This interference was due, at least in part, to the dogs receiving autotransfusions with a marked increase in their white blood count. Even Dog E (the control dog), which had an abdominal incision and received blood from a donor dog, may have developed a significant infection with a 24-hour white blood count of 36,000. Therefore, all five dogs were initially not in the best of health.

FIGURES AND TABLES

Figure 1. A special tissue-protecting device (patent pending) was developed for a high-speed handpiece to facilitate the removal of dentin without grossly macerating pulpal tissue.

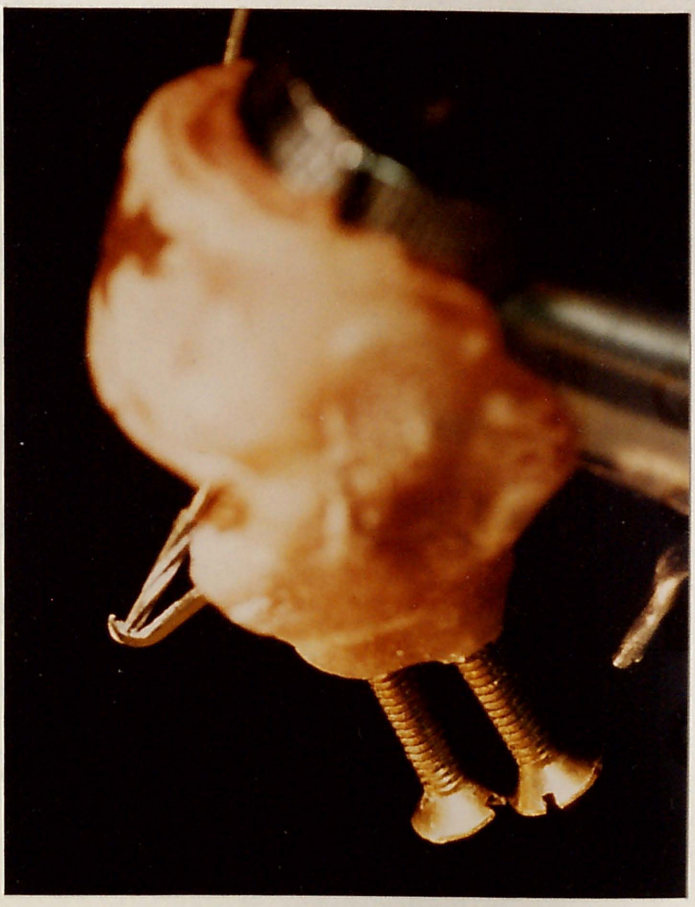
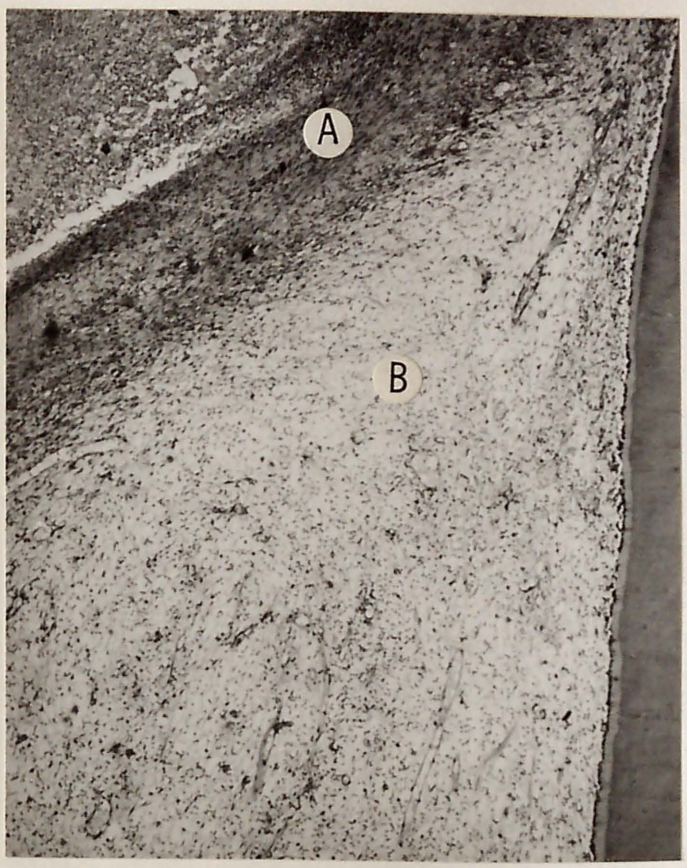


Figure 2. Representative samples of coronal pulpal biopsies without gross tissue macerations. After scalpel amputation, these tissues were delivered through buccal surface preparations from freshly extracted human third molar teeth.

Figure 3. Maxillary right first permanent molar 21 days after pulpotomy. The pulp tissue at the amputation site demonstrates a heavy inflammatory infiltrate consisting mainly of lymphocytes and polymorphonuclear neutrophils (A) within an oblique band of fibrous connective tissue which formed to wall off the infiltrate. A mild inflammatory response (B) is below the oblique band of fibrous connective tissue. (Original magnification, X 35)

Figure 4. Maxillary left first permanent molar 36 days after pulpotomy. The occlusal portion of the root canal (A) shows a moderate infiltration of round cells, followed by a mild inflammatory response (B) at the bottom half of the photomicrograph. The odontoblastic layer has been pulled away from the canal wall as a result of an artifact formed by cutting the tissue section. (Original magnification, X 35)


A
B

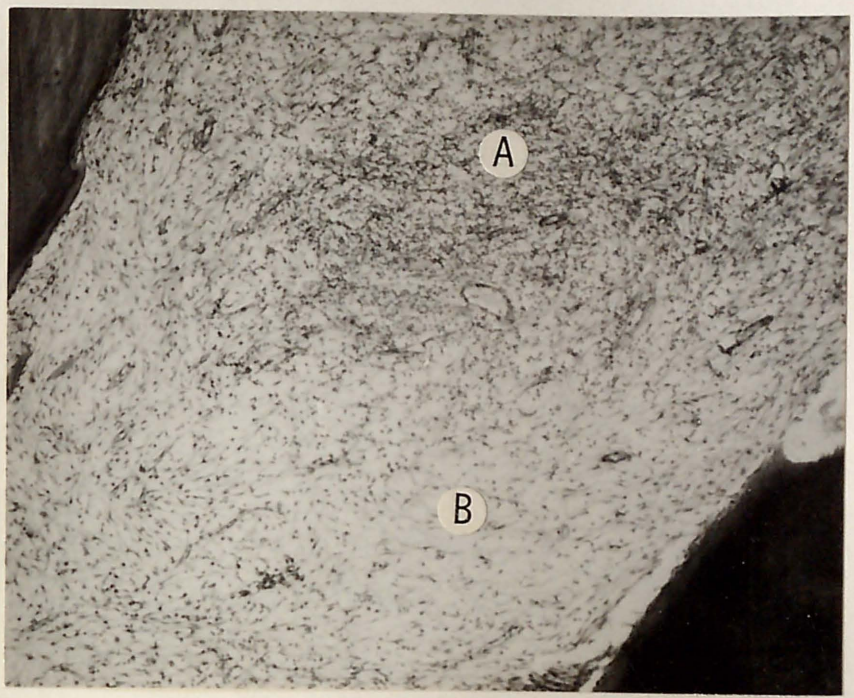

A
B

Figure 5. Resin diaphragm with plastic pin.

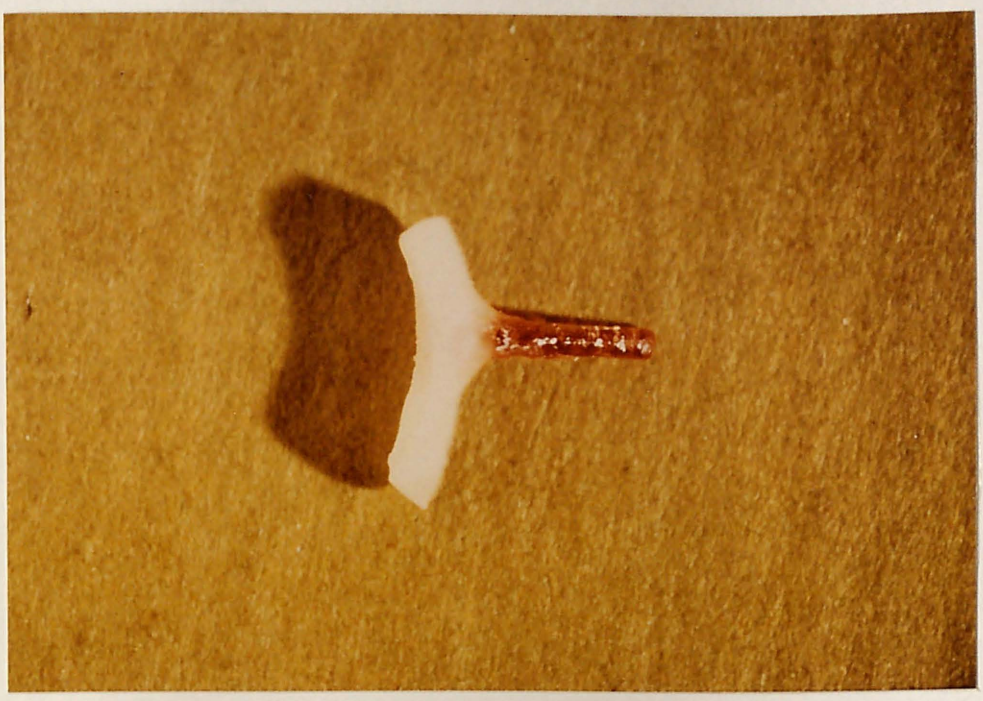


Figure 6.1. Leakage of Ca^{45} for the complete extent of the cervical and occlusal walls of the buccal Intermediate Restorative Material restoration. This penetration was considered moderate and significant.

Figure 6.2. Slight leakage of the radioisotope at the cervical and occlusal margins of the Dispersalloy buccal restoration (with Copalite cavity varnish).

Figure 6.3. Autoradiograph of the buccal cavity preparation restored with Cavit-G demonstrating severe marginal leakage. The radioisotope penetrated the full extent of the cervical and occlusal cavity walls including the buccal wall next to the resin diaphragm. In addition, the isotope completely penetrated the Cavit-G material.

Figure 6.4. Severe leakage of Ca^{45} occurred at the margins of cavity preparation restored with Durelon cement. The isotope penetrated the full extent of the cervical, occlusal, and buccal walls of the cavity preparation and completely surrounded the resin diaphragm.


6.1


6.2


6.3


6.4

Figure 7. Top. A preformed stainless steel wire mesh diaphragm with welded orthodontic bracket. Bottom. Preformed diaphragm after a thin portion of Nuva-fil has been polymerized to the wire mesh in readiness for labeling and sterilization.

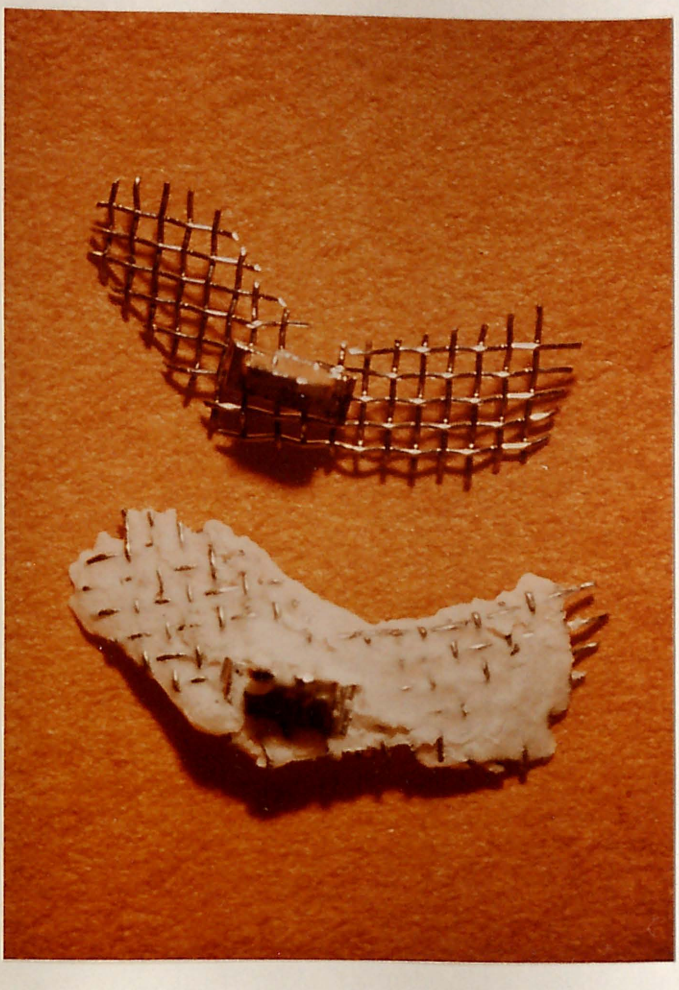


Figure 8. A special tissue-protecting device (patent pending) was designed for a surgical handpiece to facilitate the removal of dentin without grossly macerating pulpal tissue.

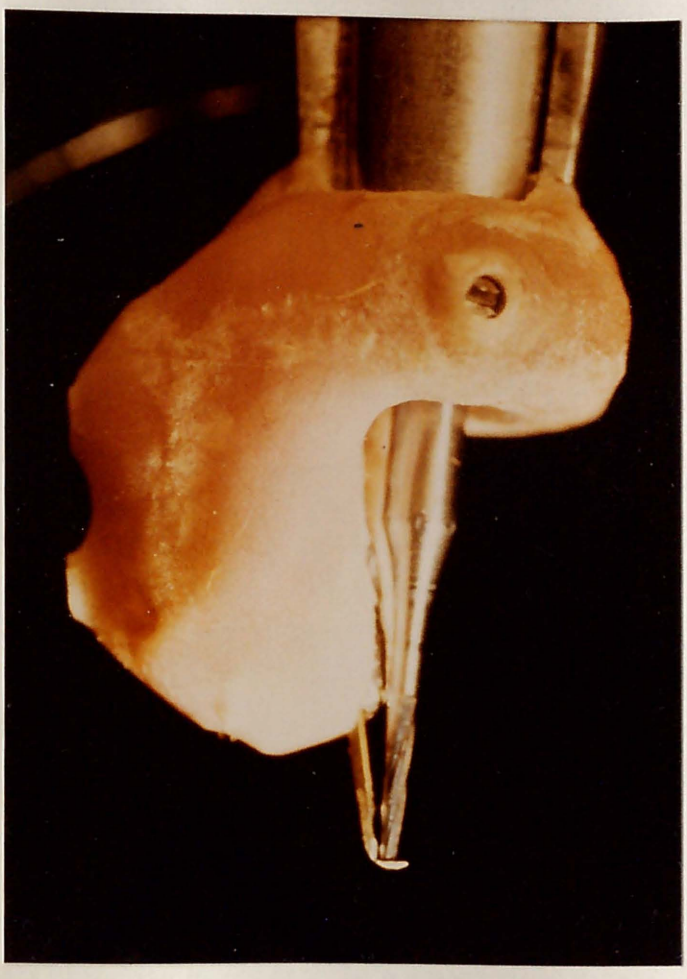
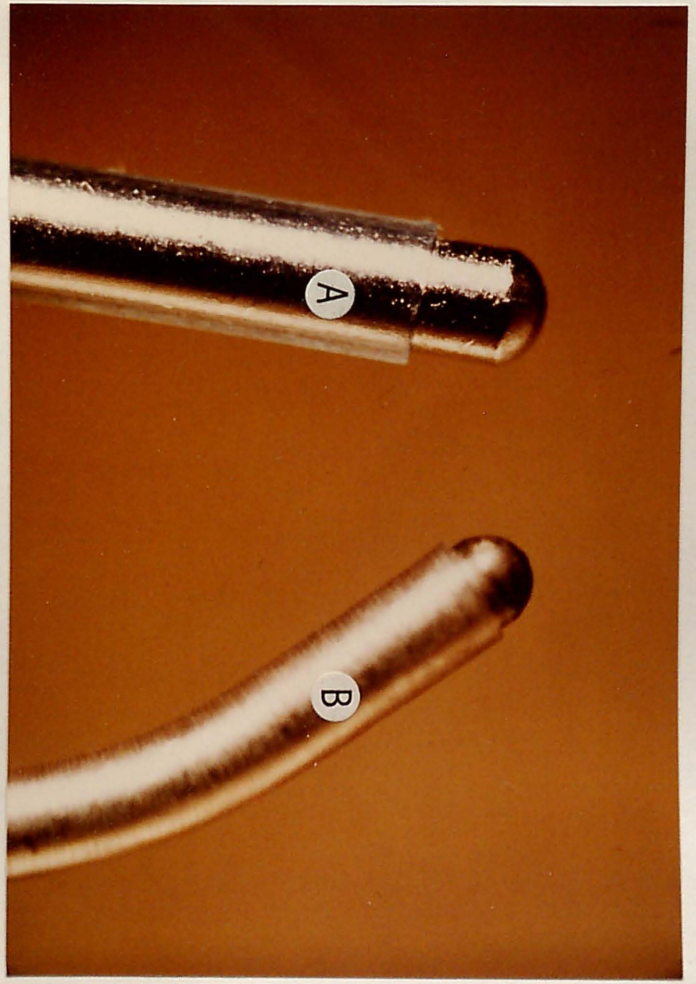


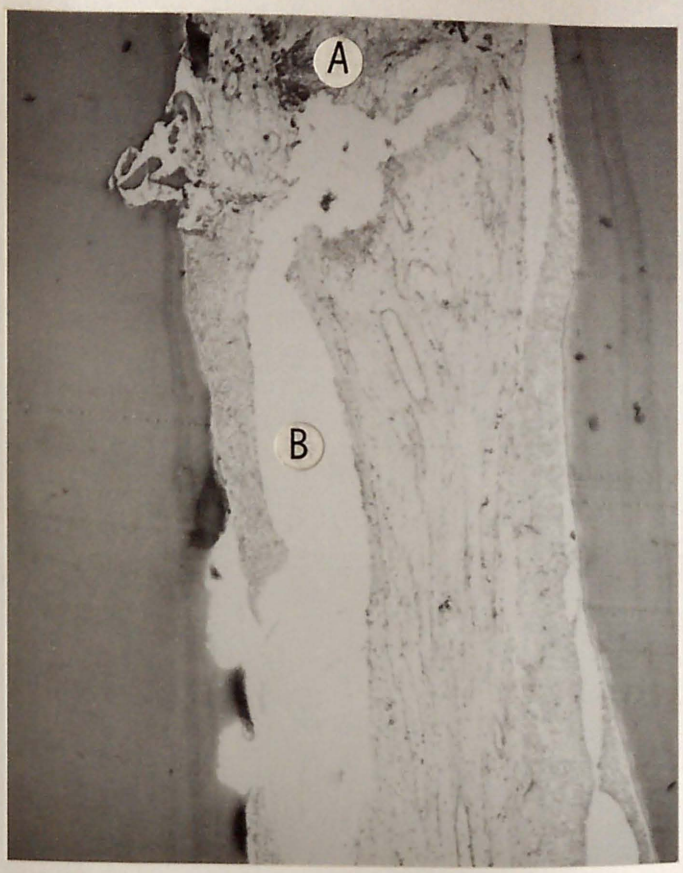
Figure 9. Cryoextractors used to deliver pulpal biopsies through buccal surface preparations.
Top: (A) Cryophake and (B) Microphake.
Bottom: (A) Cryophake curved tip, 2 millimeter diameter and (B) Microphake curved tip, 1.5 millimeter diameter.


A
B


CRYOPHAKE®
Developed by R. David Sudarsky, M.D.
CAUTION: Contents under pressure.
DO NOT AUTOCLAVE. Do not puncture.
A
microphake®
B

Figure 10. Maxillary left permanent premolar$_4$ (Dog A) 14 days after pulpotomy demonstrating coagulation necrosis. Pulpal tissue at the amputation site (A) exhibited clumps of erythrocytes. The tissue below the amputation site (B) had very loosely connected collagen fibers, with a few scattered fibroblasts and with an absence of intracellular details, and the odontoblastic layer was disrupted with an absence of odontoblasts. (Original magnification, X 35)

Figure 11. Maxillary left permanent premolar$_4$ (Dog E) 21 days after pulpotomy. In the area of the amputation site (A), a mild inflammatory infiltrate was present, the predentin layer was absent, and the odontoblastic layer was disrupted. Beneath the mild inflammatory area, the pulpal tissue (B) demonstrates abnormally loose connective tissue and scalloping of the dentinal wall. The area farther apically (C) demonstrates normal pulpal tissue. (Original magnification, X 35)


A
B

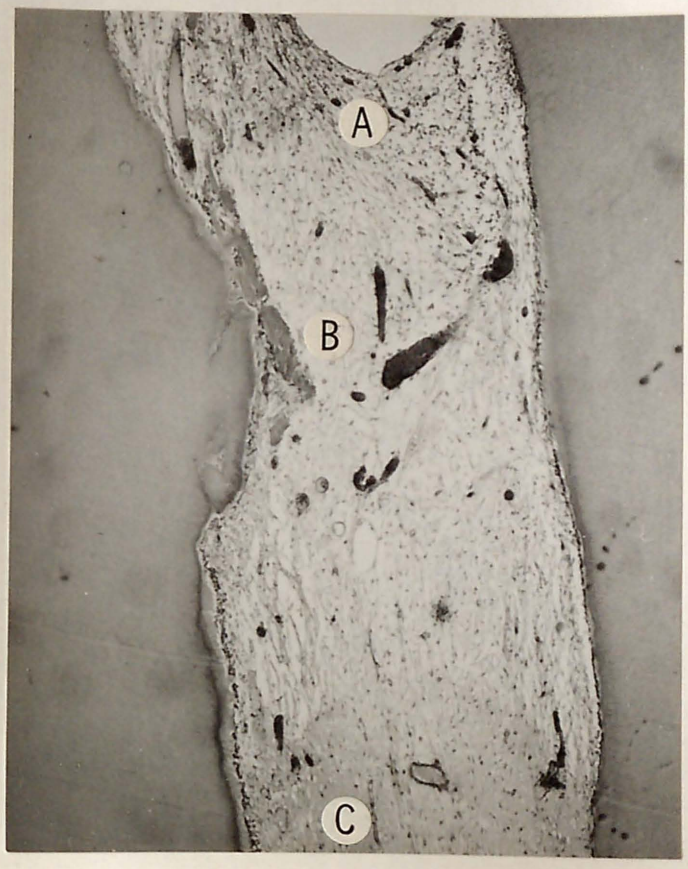
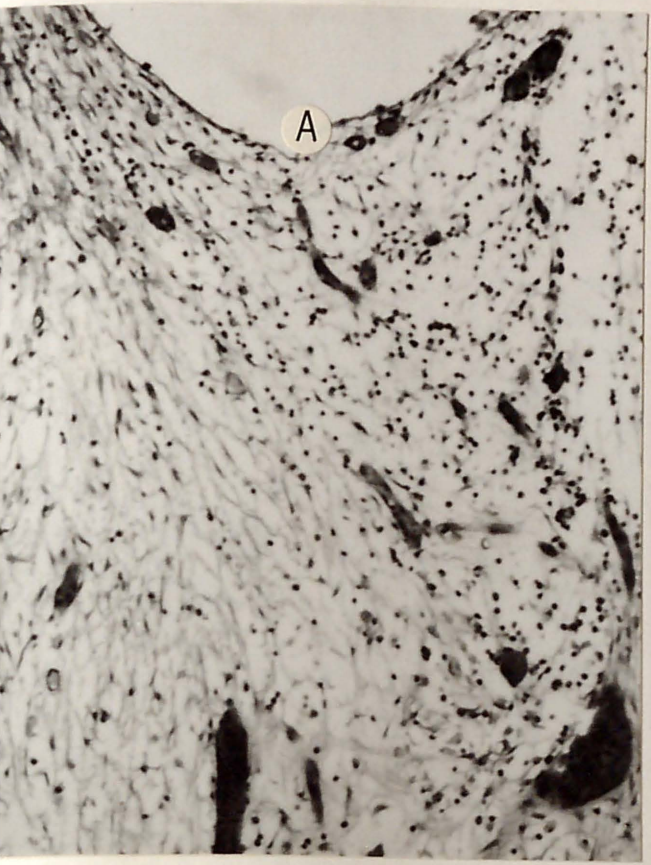
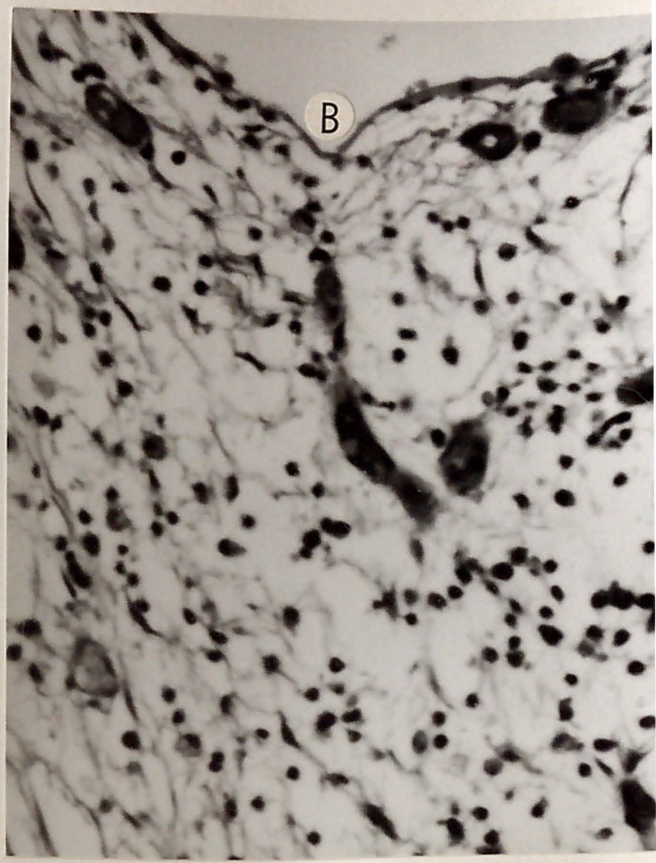

A
B
C

Figure 12. Amputation site for Dog E. A (Original magnification, X 100) and B (Original magnification, X 250) demonstrate mild inflammatory infiltrate, capillaries filled with erythrocytes, and very loose connective tissue.

Figure 13. Maxillary right permanent premolar$_4$ (Control) for Dog E. Normal-appearing pulpal connective tissue and odontoblastic layer. Vacuolation of the odontoblastic layer is probably the result of inadequate fixation.


A


B

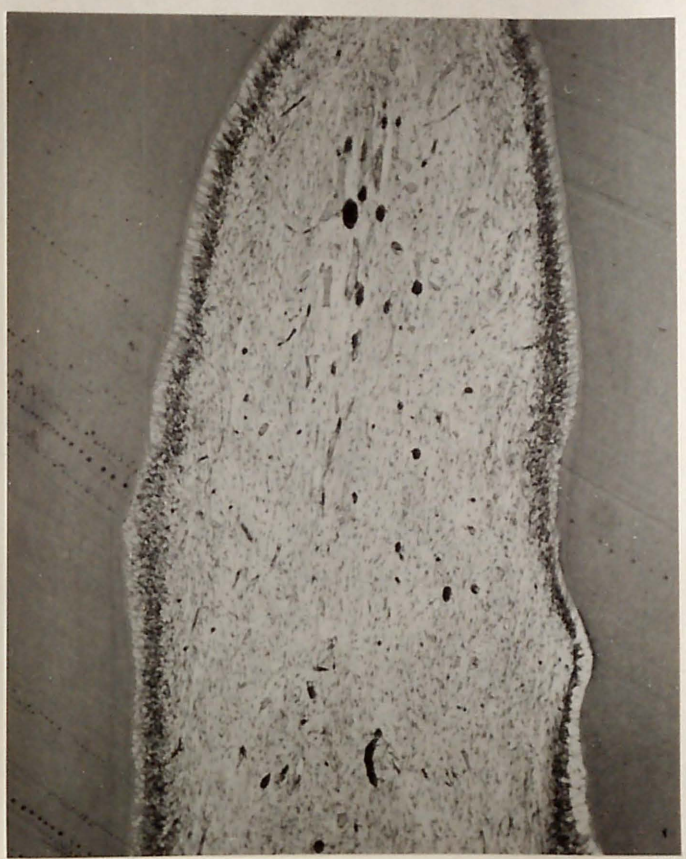
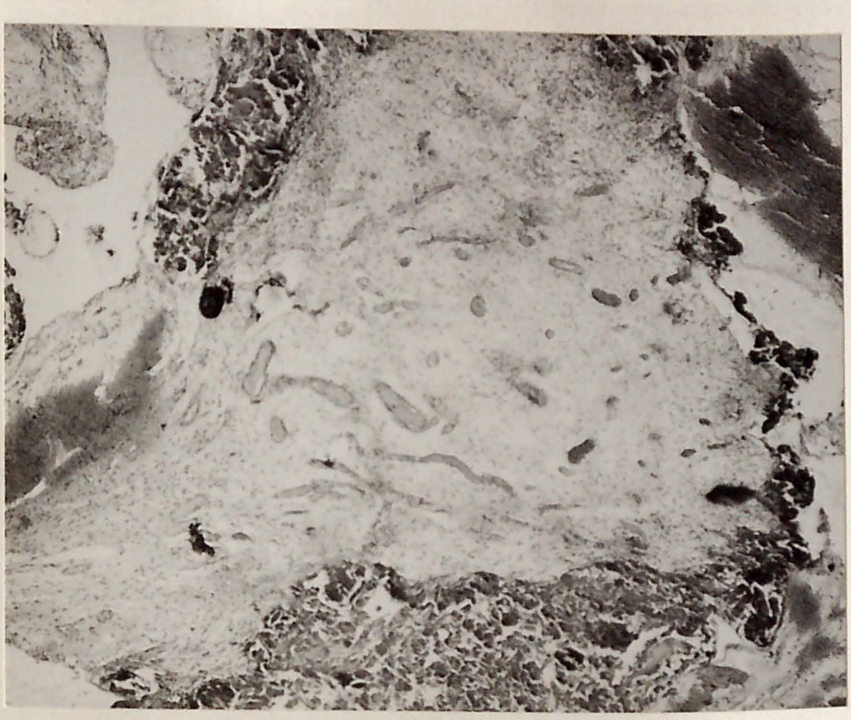


Figure 14. Pulpal biopsy for maxillary left premolar$_4$ in Dog E, demonstrating normal-appearing fibrous connective tissue with deposits of fibrin at the periphery of the specimen and disarranged odontoblasts. (Original magnification, X 35)

Figure 15. Higher magnification of the central portion of the pulpal biopsy for Dog E, showing fibroblasts, very fine fibrous connective tissue, and pulpal capillaries filled with erythrocytes. (Original magnification, X 100)



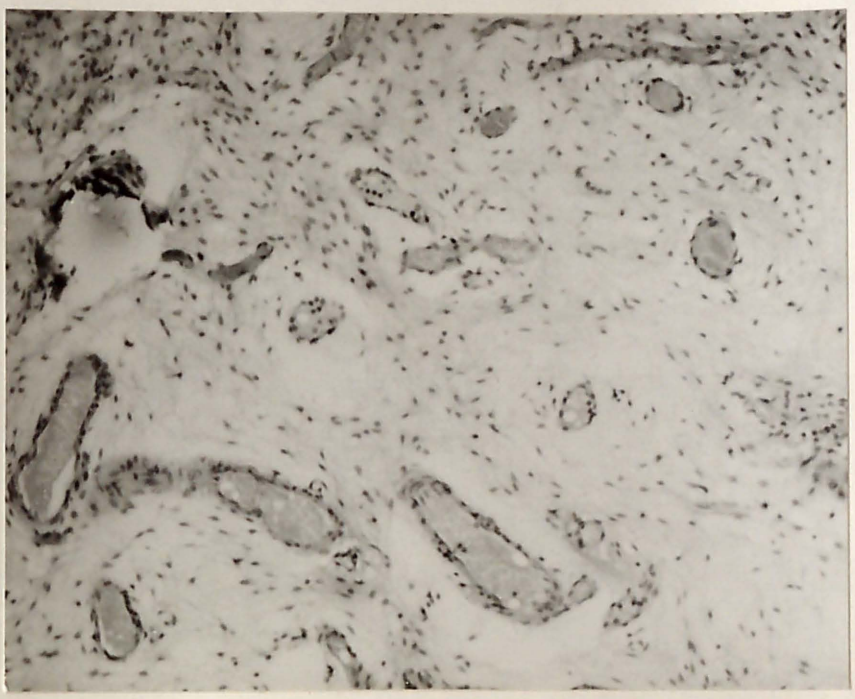


Table I. The amount of time that enamel surfaces were moistened with antimicrobial solutions correlated with resulting cultivable bacteria.

DOG	No Treatment	Betadine Solution Only	SECONDS USED FOR MAINTAINING A MOISTENED ENAMEL SURFACE WITH EACH SOLUTION[x]						
		3 Minutes	5	10	15	30	60	90	120
A						N $URPM_4$	N $LRPM_4$	N $ULPM_4$	N $LLPM_4$
B						N $URPM_4$	N $LRPM_4$	N $ULPM_4$	N $LLPM_4$
C	P $LRPM_4$	P $LRPM_4$			N URM_1	N $LRPM_4$	N $URPM_4$	N $ULPM_4$	N $LLPM_4$
D	P $LRPM_4$ P $ULPM_4$	P $LRPM_4$ P $ULPM_4$			N URC N ULC	N $LRPM_4$	N $URPM_4$	N $ULPM_4$	N $LLPM_4$
E	P $URPM_4$ P LLM_1	P $URPM_2$ P $LLPM_3$				N LLM_1	N $ULPM_4$	N $URPM_4$	N $LRPM_4$
F			N ULC N $URPM_3$ N URM_1 N ULM_1	N $ULPM_3$ P^t $LLPM_3$ N $LRPM_3$	N URC	N $URPM_4$	N $LRPM_4$	N $ULPM_4$	P^* $LLPM_4$
TOTAL	5P of 5 teeth	5P of 5 teeth	4N of 4	2N of 3	4N of 4	6N of 6	6N of 6	6N of 6	5N of 6
	Group I (control)		Group II			Group III			

[x] Combination of procedures for enamel surface in sequential order: Generalized scrub of all easily accessible enamel surfaces during a one-time three-minute period with Betadine Surgical Scrub; apply: 10 percent hydrogen peroxide, 2 percent tincture of iodine, rubber dam isolation, 30 percent hydrogen peroxide, and 2 percent tincture of iodine.

P = Positive culture. N = Negative culture.

$URPM_4$ = Designates dog's tooth (i.e. Maxillary Right $Premolar_4$).

P^* = Positive culture of gram-positive rods with growth characteristics like the genus Bacillus (a common air contaminant of microbial cultures).

P^t = Positive culture of gram-positive coccus and gram-positive rods.

Culture Taken After Following Procedure	Table II. Results of Establishing and Maintaining Tooth Structures Free of Cultivable Bacteria				
	Culture Results of Tooth Structures				
	Dog A	Dog B	Dog C	Dog D	Dog E
Disinfection of Tooth Surface	Not Cultured	Negative	Negative	Negative	Negative
Initial Preparation	Positive	Positive	Negative	Negative	Negative
Diaphragm Adapted	Positive (Gram-positive)	Negative	Negative	Negative	Negative
Completion of Second Preparation	Positive (Gram-positive)	Positive	Negative	Negative	Positive (Gram-negative rods)
Pulpal Excision and Blood Clot	Negative	Positive	Negative	Negative	Negative
Diaphragm Cemented	Negative	Negative	Negative	Negative	Negative

DISCUSSION

The successful result of freeing the tooth surface of cultivable bacteria that was recorded in Experiment Number Three (*In Vivo* Procedures for Rendering the Tooth Surface Free of Cultivable Bacteria) was replicated in the main investigation. Experiment Number Three demonstrated that the tooth surface could be rendered free of cultivable bacteria, even if the enamel was kept moist with the antimicrobial agents for only five seconds. Nevertheless, a 30-second application of each agent was chosen for the main study. Attentiveness to the details of ensuring that sterile operating room techniques were precisely followed resulted in a failure to culture the tooth surface for Dog A after the antimicrobial treatments. However, since the tooth surfaces were rendered free of cultivable bacteria in Experiment Number Three, and since consistently negative cultures following the same procedures were obtained in the other four dogs, it seems reasonable to assume a similar result for Dog A.

Even though sterile operating room procedures were followed in the pilot study (Experiment Number Two: *In Vivo* Trials for New Pulpotomy Procedures), the following additional measures to ensure the use of a sterile technique were taken for the main investigation: The operating room team received a review of instructions and practice sessions on sterile operating room techniques. A sterile pediatric laparotomy drape was used to completely cover the animal except the head. A

sterile plastic surgical drape[a] (with a small aperture) became an addition to the antimicrobial regimen. Shoe covers were worn by all members of the operating room team. Surgical handpieces that exhaust away from the operating field were used in place of the conventional high-speed handpiece.

An interpretation of the results in maintaining the tooth structure free of cultivable bacteria during the operative procedures follows. The three negative cultures following the initial cavity preparations for Dogs C, D, and E, as compared to positive cultures for Dogs A and B, may have been due to increased proficiency of the operating team in applying the Steri-Drape over the dog's face and whiskers in the antimicrobial procedures for the animals used in the latter part of the study. Even though Experiment Number Four (The Effect of Handpiece Operation on Growth of Cultivable Bacteria) indicated that using a surgical handpiece instead of a conventional high-speed handpiece decreased the likelihood of introducing bacteria at the operating site, it is possible that the rotation of the sterile drill with nothing exhausting onto the field of operation produced an aerosol of bacteria. The cleanliness of the floor, furnishings, and room air at the Wishard Memorial Hospital Animal Surgery was open to question.

Cultures of the buccal floor of the initial cavity preparation following the diaphragm adaptation demonstrated that Dogs C, D, and E maintained negative results. Dog B changed from a positive to a negative culture and Dog A maintained a positive result. No methods are known to exist for sterilizing the filled resin (Nuva-fil) without

---

[a] Steri-Drape, Surgical Products Division, 3M Company, St. Paul, MN

changing its physical properties. Therefore, preliminary trials prior to this investigation were conducted by inoculating filled resin (Nuva-fil) into thioglycollate medium. These tests indicated that cultures of the unpolymerized resin paste did not result in bacterial growth. It is conceivable that the physical application of the resin diaphragm and the subsequent polymerization with the ultraviolet light contributed to the negative culture in Dog B.

Cultures taken of the buccal wall of the second (inner) preparation resulted in a sustained negative culture for Dogs C and D. The positive cultures obtained in Dogs A, B, and E may have resulted from the operation of the handpiece generating a bacterial aerosol in something less than a clean operating room.

Since Brown and Brenn-stained sections demonstrated an absence of bacteria in the pulp chamber sections (area of pulpal amputation site) for Dogs A, C, and E, these findings correlate with the negative results obtained from the cultures taken for these animals following pulpal excision and formation of a blood clot (Table II). In addition, the positive culture obtained for Dog B following pulpal excision and blood clot formation correlates with the positive evidence of a small number of bacteria located in clumps of debris on the cervical dentinal ledge of the preparation. Also, Brown and Brenn-stained sections of the pulp chamber for Dog B demonstrated evidence of small crystalline particles which possibly could have been fragments from the Nuva-fil portion of the diaphragm.

Brown and Brenn-stained sections of the pulpal tissue in the root canals supported the sterile operating room technique. This evaluation was based on the absence of bacteria in the tissue sections for Dogs C

and E and on the rather meager suggestive evidence of a small number of bacteria in the pulpal tissues of Dogs A and B. On the other hand, these results suggest that the complete elimination of microbial contamination at an intraoral operative site is very difficult to achieve on a consistent basis. It is assumed that if sterile operating room techniques had not been used, more of the test dogs would have exhibited more bacteria in the pulp tissue.

It is interesting that all cavity preparations and amputation sites, except in Dog B, demonstrated negative cultures after the blood clot formation. The change from positive cultures for Dogs A and E, which were present following the completion of the second (inner) preparation, may have resulted from the physical cleansing of the physiologic balanced salt solution which was used to irrigate the amputation site.

In all five dogs, the cultures of the cemented resin diaphragms revealed negative results for bacterial growth. Therefore, in Dog B, the positive culture after the blood clot changed to a negative culture of the cemented diaphragm, which may have been promoted by any antimicrobial properties of the cementing substance used to secure the diaphragm in place.

It was gratifying that cultures of the tooth structures in Dogs C and D maintained negative signs of bacterial growth after each of the five major operative procedures.

This study confirmed the preliminary findings of Experiment Number One (Use of a Tissue-Protecting Device to Remove Dentin Over Pulpal

Tissue) and supported the pilot study (Experiment Number Two: *In Vivo* Trials for New Pulpotomy Procedures) in the effective removal of dentin. The tissue-protecting device performed successfully in all five animals for the removal of buccal dentin over the coronal portion of the pulp without grossly macerating pulpal tissue. This study further emphasized the finding of the pilot study in that the tissue-protecting device performed more efficiently after only an extremely thin layer of dentin remained between the buccal wall and the pulpal tissue. As in the case of the pilot study, the pulpal tissue could not be uncovered without producing slight hemorrhage from the pulp.

Replicating the results in Experiments One and Two, a scalpel severance of the coronal portion of the pulpal tissue was able to be performed in this study. For the main investigation, a Beaver 59M eye knife proved to be of adequate size, sharpness, and rigidity to sever the pulpal tissue.

The use of sterile cryoextractors was effective in the delivery of the excised coronal portion of the pulp through the buccal preparation. However, in each dog, as in the pilot study, the excised pulpal tissue was delivered in fragments. This was different from the situation of Experiment Number One (Use of a Tissue-Protecting Device to Remove Dentin Over Pulpal Tissue) which was an *in vitro* study where the pulpal tissue was chilled and without blood pressure. The cryoextractor usually was effective in delivering the largest fragment of excised pulpal tissue and sometimes one or two smaller portions. Unlike Experiment Number One, the coronal pulpal tissue was never able to delivered in toto.

Using a performed sterile resin-stainless steel wire diaphragm was a marked improvement over the frustration of forming the stainless steel plate diaphragm in the pilot study. The diaphragm in this study rested against the dentinal ledge formed by constructing the second preparation. Occasionally, an insufficient ledge of dentin was present in the occlusal portion of the preparation and the diaphragm rested against the lingual wall of the pulp chamber which, of course, was free of pulpal tissue. In each dog, the diaphragm was able to be cemented to place without touching the pulpal amputation site. The diaphragm was effectively cemented to place with polycarboxylate cement by painting the cement on the periphery of the diaphragm with a very fine brush. The diaphragm remained securely in place for a period of 21 days with the exception of Dog A (the Animal Surgery required terminating the use of this dog after 14 days) and in Dog D (the Animal Surgery mistakenly sacrificed this one 15 days after the pulpotomy procedure).

In all animals (except Dog D, for which data were not available), the amalgam restoration was properly retained and clinically the amalgam maintained an adequate relationship with the cavosurface margins of the preparation.

As identified in the pilot study following the pulpal amputation, blood clot formation, and cementation of the diaphragm, slight oozing of a serous-sanguinous exudate at the periphery of the diaphragm presented somewhat of a problem during the setting of the cement. The presence of the exudate from the wound was not unexpected, since no

medicaments were applied to the amputation site, with the exception of the physiologic balanced salt solution which was used to irrigate away tissue fragments and dentin chips.

Hassan, Van Huysen, and Gilmore[99] studied deep cavities with superficial pulp exposures in the teeth of dogs. With the aid of the dissecting microscope, "dental pulp fluid" was observed accumulating on the cavity floor which was proved by histologic section to be a minute non-hemorrhagic pulp exposure. These investigators concluded that the "dental pulp fluid" was under positive pressure. With the injection of Evans Blue dye and Tetracycline, the "dental pulp fluid" excaping from the pulp exposures indicated that this fluid came from the extravascular tissue or connective tissue ground substance.

It is possible that the serous exudate, which continued to flow after hemostasis in the main study, may have been "dental pulp fluid".

The blood transfusions performed by the medical research team prior to this study correlate well with the histologic sections. In Dog E (the control dog of the medical research project) the pulpal reaction to the amputation was a moderate to mild inflammatory response that was thought to be reversible. On the other hand, Dogs A, B, and C (Dog D was sacrificed inadvertently by the Wishard Memorial Hospital Animal Surgery) demonstrated pulpal necrosis which could be correlated with the questionable health of the dogs. The immune system was certainly affected as an example of the increased value in the white blood cell count. The essence of this study shows that the surface of a dog's tooth can be rendered free of cultivable bacteria and the operative site maintained in this condition throughout pulpotomy procedures, buccal dentin can be removed to expose the coronal portion of the pulp

without grossly macerating pulpal tissue, a scalpel severance of the pulp can be performed so that the coronal portion can be removed from the chamber, and a sterile diaphragm can be placed without touching the pulpal amputation site. Under these conditions a potential exists for satisfactory wound healing, with possible implications for clinical application.

SUMMARY AND CONCLUSIONS

Analysis of traditional pulpotomy procedures and consideration for improving this treatment resulted in conducting a series of five preliminary experiments with direct relationship to performing the main study.

An *in vitro* study using freshly extracted human third molars demonstrated that the following procedures could be carried out successfully and consistently: removing buccal dentin over the coronal portion of the pulp without macerating pulpal tissue, performing a smooth incision of the pulpal tissue, and delivering the coronal pulpal tissue in toto from the pulp chamber.

New pulpotomy procedures were conducted as *in vivo* trials in a pilot study for the main investigation. Using the dog as the animal model, a tissue-protecting device effectively removed the buccal dentin over the coronal pulp without gross maceration of tissue. The coronal pulp was exicsed with an ophthalmic knife from a buccal entry and was delivered in fragments. Histologic sections revealed that a heavy inflammatory infiltrate was present at the amputation site. It was theorized that the invasion of inflammatory cells resulted from the introduction of bacteria at the operation site or from leakage of the restoration's cavity seal, rather than as a response to the surgical trauma of the pulpal tissue.

The third experiment was an *in vivo* study of the correlation between the increments of time used to maintain enamel surfaces moistened with test solutions and the resultant positive or negative

cultures for the presence of bacteria. Enamel surfaces in dogs were rendered free of cultivable bacteria, whether the application of four solutions and the rubber dam with maintenance of a moist surface for each solution lasted only 5 seconds or for periods up to 120 seconds of contact time for each solution.

The fourth experiment was performed to verify that a surgical handpiece should be used in sterile surgery in place of a conventional high-speed handpiece. The operation of surgical handpieces did not produce cultivable bacteria at the operation site, whereas the conventional high-speed handpiece demonstrated a positive culture. Although considerable handpiece speed had to be relinquished with the use of a surgical handpiece, removing the capability of a handpiece to introduce bacteria at the site of operation was an important factor in the design of the main investigation.

The fifth experiment compared the leakage capabilities of several materials by the use of autoradiographs. Amalgam with two coats of cavity varnish proved to have the least leakage capability as compared with IRM, Durelon, and Cavit-G. Since Cavit-G was used in the pilot study as the outer cavity restoration and heavy inflammatory infiltrate occurred in the pulpal tissue, amalgam and cavity varnish were used to restore the preparation in the main study.

The purpose of the main study was to determine the feasibility of performing a sterile scalpel excision of coronal pulpal tissue and to evaluate wound healing after a shield had been placed to prevent all substances from touching the blood clot at the amputation site.

Using sterile operating room procedures, four of the five study teeth were rendered free of cultivable bacteria after antimicrobial treatments were followed, which fully supported the initial portion of the first hypothesis (the surface of a dog's tooth can be rendered free of cultivable bacteria and the operative site maintained in this condition throughout pulpotomy procedures). These findings replicated the results of the third preliminary experiment involving 45 teeth, which demonstrated that the sterilizing procedures for tooth enamel in dogs were effective significantly beyond the 0.001 level as compared to a control group. Furthermore, in the main study, negative cultures were obtained during 72 percent of the trials following the five major operative procedures on the study teeth. This included 80 percent negative cultures for bacteria after the pulpal excision and blood clot formation, and 100 percent freedom from cultivable bacteria after the resin diaphragm was cemented into place.

Using an operating microscope, the buccal dentin over the coronal portion of the pulp was consistently able to be removed without grossly macerating the pulpal tissue. The coronal portion of pulpal tissue was amputated with a scalpel severance and removed through a buccal preparation in large fragments. After blood clot formation, closure of the cavity preparation was able to be initiated without applying medicaments to the amputation site. The successful completion of dentin removal with a tissue-protecting device, straight excision of the coronal portion of pulp, and the absence of applications of medicaments to the blood clot agreed with findings from the three experimental teeth in the pilot study (Preliminary Experiment Number Two: <u>In Vivo</u> Trials for New Pulpotomy Procedures).

Resin-stainless steel diaphragms were constructed under sterile operating room conditions and secured in buccal preparations without touching the pulpal amputations for a convalescent period of 14 to 21 days. The diaphragm fabrication was improved from the pilot study and continued to be successful in providing a buccal wall for closure with an amalgam restoration.

All five dogs demonstrated no signs of ill health or discomfort during the posttreatment period of this study, and they continued to consume regular rations of food. At the time of extraction, there was no evidence of gingival pathosis or tooth mobility. These findings support the results of the pilot study.

Histologic sections of the pulp tissue in the root canals demonstrated satisfactory wound healing limited to a moderate and mild inflammatory infiltrate in the one animal which did not receive an autotransfusion. This 21-day specimen was compared to a control un-operated tooth from the same animal. The remaining three dogs revealed pulpal necrosis which was attributed to an interference in the immune system of the study animals by previous autotransfusion research.

In sum, this study demonstrates that the enamel surface can be rendered free of cultivable bacteria in the dog and the operative site can be maintained in this condition throughout pulpotomy procedures. Also, the coronal portion of the pulp can be removed without grossly macerating the pulp, a scalpel severance of the pulp can be performed so that the coronal portion can be removed from the pulp chamber, and a diaphragm can be constructed under sterile operating room conditions

and secured in place without touching the pulpal amputation site. Under these conditions there appears to be a potential for satisfactory wound healing, with possible implications for the clinical situation.

REFERENCES

1. Wunder, J.A.; Briner, W.W.; and Calkins, G.P.: Identification of the cultivable bacteria in dental plaque from the beagle dog. J Dent Res 55:1097-1102, 1976.

2. Courant, P.R.; Saxe, S.R.; Nash, L.; and Roddy, S.: Sulcular bacteria in the beagle dog. Periodontics 6:250-252, 1968.

3. Birch, R.H., and Melville, T.H.: Preliminary sterilisation of the endodontic field. Br Dent J 3:362-363, 1961.

4. Moller, A.J.R.: Microbiological examination of root canals and periapical tissues of human teeth. Odont Tskr 74:3-68, 1966.

5. Griffee, M.B.: The Relationship of Bacteroides melaninogenicus to Symptoms of Pulp Necrosis. Master's Thesis, Indiana University School of Dentistry, 1979.

6. Ahmed, F.I.K., and Russell, C.: Sterilisation of teeth for homogenous transplantation. Br J Oral Surg 14:143-149, 1976.

7. Melville, T.H., and Birch, R.H.: Preliminary sterilisation of the endodontic field. Br Dent J 110:313-314, 1961.

8. Armstrong, R.L.; Patterson, S.S.; Kafrawy, A.H.; and Feltman, E.M.: Comparison of Dycal and formocresol pulpotomies in young permanent teeth in monkeys. Oral Surg 48:160-168, 1979.

9. Schroder, U., and Granath, L.E.: On internal dentine resorption in deciduous molars treated by pulpotomy and capped with calcium hydroxide. Odont Revy 22:179-188, 1971.

10. Magnusson, B.: Attempts to predict prognosis of pulpotomy in primary molars. Bacteriologic and histologic examination. Scand J Dent Res 78:232-240, 1970.

11. Engstrom, B., and Spangberg, L.: Wound healing after partial pulpectomy. A histologic study performed on contralateral tooth pairs. Odont Tskr 75:5-18, 1967.

12. Sandler, E.S.; Frankl, S.N.; and Ruben, M.P.: The histological response of the dental pulp to cresatin. J Dent Child 38:49-58, 1971.

13. Kouri, E.M.; Matthews, J.L.; and Taylor, P.P.: Epinephrine in pulpotomy. J Dent Child 36:123-128, 1969.

14. Sciaky, I., and Pisanti, S.: Localization of calcium placed over amputated pulps in dog's teeth. J Dent Res 39:1128-1132, 1960.

15. Seltzer, S., and Bender, I.B.: Some influences affecting repair of the exposed pulp of dogs' teeth. J Dent Res 37:678-687, 1958.

16. Magnusson, B.O.: Therapeutic pulpotomies in primary molars with the formocresol technique. A clinical and histological follow-up. Acta Odontol Scand 36:157-165, 1978.

17. Spedding, R.H.: The one-appointment formocresol pulpotomy for primary teeth. J Tenn S Dent Assoc 48:263-270, 1968.

18. Corpron, R.E.: Pulpal therapy for primary teeth. J Mich Dent Assoc 50:341-346, 1968.

19. Hansen, H.P.; Ravn, J.J.; and Ulrich, D.: Vital pulpotomy in primary molars. A clinical and histologic investigation of the effect of zinc oxide-eugenol cement and Ledermix. Scand J Dent Res 79:13-23, 1971.

20. Stark, M.M.; Nicholson, R.J.; Soelberg, K.; and Appel, P.: Hemorrhage control in the dental pulp with the use of resorbable oxycellulose. J S Calif Dent Assoc 36:491-493, 1968.

21. Frigoletto, R.L.: Pulp therapy in pedodontics. J Am Dent Assoc 86:1344-1348, 1973.

22. Andrioni, J.N., and Russo, M.D.C.: Evaluation of vital and devitalizing methods for pulpotomy in deciduous teeth. Rev Fac Odontol Aracatuba 3:291-302, 1974.

23. Schroder U.: A 2-year follow-up of primary molars, pulpotomized with a gentle technique and capped with calcium hydroxide. Scand J Dent Res 86:273-278, 1978.

24. Britton, A.S.: Total coronal pulpotomy of the vital permanent molar. Proc Br Paedod Soc 6:15-18, 1976.

25. Bergenholtz, G.: Effect of bacterial products on inflammatory reactions in the dental pulp. Scand J Dent Res 85:122-129, 1977.

26. Brown, R.V.: Bacterial aerosols generated by ultra high-speed cutting instruments. J Dent Child 32:112-117, 1965.

27. Kreshover, S.J., and Bevelander, G.: Histopathology of the dental pulp of dogs following exposure. J Dent Res 27:467-479, 1948.

28. Granath, L.E., and Hagman, G.: Experimental pulpotomy in human bicuspids with reference to cutting technique. Acta Odontol Scand 29:155-163, 1971.

29. Schroder, U.: Effect of an extra-pulpal blood clot on healing following experimental pulpotomy and capping with calcium hydroxide. Odont Revy 24:257-268, 1973.

30. Schroder, U., and Granath, L.E.: Early reaction of intact human teeth to calcium hydroxide following experimental pulpotomy and its significance to the development of hard tissue barrier. Odont Revy 22:379-396, 1971.

31. Davis, W.C.: Structural changes within the pulp canals of teeth following partial pulp removal. Dent Cosmos 63:1198, 1921.

32. Davis, W.C.: Structural changes within the pulp canals of teeth following partial pulp removal. Dent Summary 41:482-492, 1921.

33. Grove, C.J.: Some causes for periapical infections with the pathology. J Natl Dent Assoc 6:669-680, 1919.

34. Davis, W.C.: Pulpotomy vs. pulp-extirpation. Dent Items of Interest 44:81-101, 1922.

35. Davis, W.C.: Pulpotomy with special reference to partial pulpectomy. Dent Items of Interest 44:721-730, 1922.

36. Davis, W.C.: Partial pulpectomy. Dent Items of Interest 44:801-809, 1922.

37. Davis, W.C.: Pulpotomy, pulpectomy, and partial pulpectomy. 4th ed.: Essentials of Operative Dentistry. St. Louis, C.V. Mosby Company, p. 247-297, 1923.

38. Nyborg, H., and Halling, A.: Amputation instruments for partial pulp extirpation. II. A comparison between the efficiency of the Antheos root canal reamer and the Hedstrom file with cut tip. Odont Tskr 71:277-283, 1963.

39. Mejare, B.; Nyborg, H.; and Palmkvist, E.: Amputation instruments for partial pulp extirpation. III. A comparison between the efficiency of the Hedstrom file with cut tip and an experimental instrument. Odont Revy 21:63-69, 1970.

40. Baume, L.J.; Holz, J.; and Risk, L.B.: Radicular pulpotomy for category III pulps. Part II. Instrumentation and technique. J Prosthet Dent 25:525-531, 1971.

41. Baume, L.J.; Holz, J.; and Risk, L.B.: Radicular pulpotomy of category III pulps. Part III. Histologic evaluation. J Prosthet Dent 26:649-657, 1971.

42. Anderman, I.I.: Pedodontic electrosurgery. Quintessence Int 8:31-37, 1977.

43. Yakushiji, M.: Vital pulpotomy on unmatured permanent teeth. Shikai Tenbo 59:267-275, 1982.

44. Hermann, B.W.: Cited by Castagnola, L., and Orlay, G.H.: Direct capping of the pulp and vital amputation. Br Dent J 88:324-330, 1950.

45. Masterton, J.B.: Inherent healing potential of the dental pulp. Br Dent J 120:430-436, 1966.

46. Massler, M: Preserving the exposed pulp: a review. J Pedod 2:217-227, 1978.

47. Johnson, R.; Yaari, A.; Berkowitz, R.; and Currier, G.F.: Techniques of pulp therapy for primary and immature permanent teeth. Compend Contin Educ Dent 1:27-35, 1980.

48. Holland R.; DeMello, W.; Nery, M.J.; DeSouza, V.; Bernabe, P.F.; and Otoboni-Filho, J.A.: Healing process of dog dental pulp after pulpotomy and pulp covering with calcium hydroxide in powder or paste form. Acta Odontol Pediatr 2:47-51, 1981.

49. Ehrmann, E.H.: Pulpotomies in traumatized and carious permanent teeth using a corticosteroid-antibiotic preparation. Int Endod J 14:149-156, 1981.

50. Morrant, G.A.: Dental instrumentation and pulpal injury. J Br Endod Soc 10:3-8, 1977.

51. Berk, H.; Krakow, A.A.; and Stanley, H.R.: Clinical situations in which amputation is preferred to pulp capping because of biologic considerations. J Am Dent Assoc 90:801-805, 1975.

52. Stanley, H.R.: The cells of the dental pulp. Oral Surg 15:849-858, 1962.

53. Gotjamanos, T.: A method for isolating an intact dental pulp from rat dentine. Arch Oral Biol 14:729-730, 1969.

54. Torneck, C.D.: Changes in the fine structure of the human dental pulp subsequent to carious exposure. J Oral Pathol 6:82-95, 1977.

55. Edelhauser, H.F.; Van Horn, D.L.; Hyndiuk, R.A.; and Schultz, R.O.: Intraocular irrigating solutions, their effect on the corneal endothelium. Arch Ophthalmol 93:648-657, 1975.

56. Edelhauser, H.F.; Van Horn, D.L.; Schultz, R.P.; and Hyndiuk, R.A.: Comparative toxicity of intraocular irrigating solutions on the corneal endothelium. Am J Ophthalmol 81:47-481, 1976.

57. Schroder, U.: Evaluation of healing following experimental pulpotomy of intact human teeth and capping with calcium hydroxide. Odont Revy 23:329-340, 1972.

58. Bahn, S.L., and Mursch, P.I.: The effects of cold on hemostasis. Oral Surg 49:294-300, 1980.

59. Phillips, R.W.: Skinner's Science of Dental Materials, 8th ed., Philadelphia, W.B. Saunders Co., 1982, p. 58.

60. Armstrong, W.D., and Simon, W.J.: Penetration of radiocalcium at the margins of filling materials, a preliminary report. J Am Dent Assoc 43:684-686, 1951.

61. Massler, M., and Ostrovsky, A.: Sealing qualities of various filling materials. J Dent Child 21:228-234, 1954.

62. Weiss, M.B.: An improved zinc oxide and eugenol cement. Ill Dent J 27:261-271, 1958.

63. Dute, H.L.; Going, R.E.; and Massler, M.: Penetration of I^{131} around and into various filling materials. J Dent Res 37:17, 1958. (Abstract)

64. Swartz, M.L.: In Vitro Studies on Marginal Leakage of Restorative Materials as Assessed by Ca^{45}. Master's Thesis, Indiana University School of Dentistry, 1959.

65. Gilmore, H.W.: A Clinical Study on the Marginal Leakage of Restorative Materials as Determined by Ca^{45}. Master's Thesis, Indiana University School of Dentistry, 1961.

66. Going, R.E.; Massler, M.; and Dute, H.L.: Marginal penetrations of dental restorations as studied by crystal violet dye and I^{131}. J Am Dent Assoc 61:285-300, 1960.

67. Swartz, M.L., and Phillips, R.W.: Influence of manipulative variables on the marginal adaptation of certain restorative materials. J Prosthet Dent 12:172-181, 1962.

68. Barber, D.; Lyell, J.; and Massler, M.: Effectiveness of copal resin varnish under amalgam restorations. J Prosthet Dent 14:533-536, 1964.

69. Dolvan, R.C.: Micromeasurement of cavity lining, using ultraviolet and reflected light and the effect of the liner on marginal penetration evaluated with Ca^{45}. J Dent Res 45:12-15, 1966.

70. Marosky, J.E.: In Vitro Studies on Marginal Leakage of Restorative Materials as Assessed by Ca^{45}. Master's Thesis, Indiana University School of Dentistry, 1975.

71. Holland, R.; Mello, W.; Souza, V.; Nery, M.J.; Bernabe, P.F.; and Filho, J.A.O.: The influence of the sealing material in the healing process of inflamed pulps capped with calcium hydroxide or zinc oxide-eugenol cement. Acta Odontol Pediatr 2:5-9, 1981.

72. Masterton, J.B.: The healing of wounds of the dental pulp. An investigation of the nature of the scar tissue and of the phenomena leading to its formation. Dent Pract 16:325-339, 1966.

73. Isermann, G.T., and Kaminski, E.J.: Pulpal response to bacteria in the dog. Oral Surg 48:353-357, 1979.

74. Isermann, G.T., and Kaminski, E.J.: Pulpal response to minimal exposure in presence of bacteria and Dycal. J Endod 5:322-327, 1979.

75. Kakehashi, S.; Stanley, H.R.; and Fitzgerald, R.J.: The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg 20:340-349, 1965.

76. Kalnins, V., and Frisbie, H.E.: The effect of dentine fragments on the healing of the exposed pulp. Arch Oral Biol 2:96-103, 1960.

77. Cabrini, R.L.; Maisto, O.A.; and Manfredi, E.E.: Internal resorption of dentine: histopathologic control of eight cases after pulp amputation and capping with calcium hydroxide. Oral Surg 10:90-96, 1957.

78. Paterson, R.C.: Bacterial contamination and the exposed pulp. Br Dent J 140:231-236, 1976.

79. McWalter, G.M.; El-Kafrawy, A.H.; and Mitchell, D.F.: Long-term study of pulp capping in monkeys with three agents. J Am Dent Assoc 93:105-110, 1976.

80. Berman, D.S., and Massler, M.: Experimental pulpotomies in rat molars. J Dent Res 37:229-242, 1958.

81. Haruyama, Y.; Asai, Y.; and Sekine, N.: Clinico-pathological observations on the healing effects of human vital pulp wounds with calcium hydroxide pastes. Bull Tokyo Dent Coll 16:199-213, 1975.

82. Kosugi, K.; Haruyama, Y.; Asai, Y.; and Sekine, N.: Clinico-pathological observations on the vital pulpotomy with or without the use of a rubber dam. Bull Tokyo Dent Coll 17:131-146, 1976.

83. Masterton, J.B.: Internal resorption of the dentine. A complication arising from unhealed pulp wounds. Br Dent J 118:241-249, 1965.

84. Smith, A.H.: Healing processes in the dental pulp. Br Dent J 19:654-660, 1898.

85. Pereira, J.C., and Stanley, H.R.: Pulp capping: influence of the exposure site on pulp healing--histologic and radiographic study in dogs' pulp. J Endod 7:213-223, 1981.

86. Bimstein, E., and Shoshan, S.: Enhanced healing of tooth-pulp wounds in the dog by enriched collagen solution as a capping agent. Arch Oral Biol 26:97-101, 1981.

87. Quigley, M.B.: Experimental exposure of hamster pulp. Oral Surg 9:1124-1131, 1956.

88. Yamada, T.: Experimental study on the influences of resected portion of the dental pulp on healing. J Osaka Dent Univ 5:106, 1971.

89. Hayashi, Y.: Ultrastructure of initial calcification in wound healing following pulpotomy. J Oral Pathol 11:174-180, 1982.

90. Stanley, H.R.: Importance of the leukocyte to dental and pulpal health. J Endod 3:334-341, 1977.

91. Brubaker, L.H.: Unsticky neutrophils. N Engl J Med 291:674-675, 1974.

92. Woehrlen, A.E., Jr.: Evaluation of techniques and materials used in pulpal therapy based on a review of the literature: part I. J Am Dent Assoc 95:1154-1158, 1977.

93. Woehrlen, A.E., Jr.: Evaluation of techniques and material used in pulpal therapy based on a review of the literature: part II. J Am Dent Assoc 96:107-112, 1978.

94. Kafrawy, A.H.: Biology of the dental pulp. Alumni Bull Sch Dent Indiana Univ Spring:4-38, 1978.

95. Donlon, W.C.: Immunology in dentistry. J Am Dent Assoc 100:220-230, 1980.

96. Olsen, R.G.: Immunology and immunopathology of domestic animals. Springfield, Charles C. Thomas, 1978, p. 5-99.

97. Adamkiewicz, V.W.; Pekovic, D.D.; and Mascres, C.: Allergies of the dental pulp. Oral Surg 46:843-853, 1978.

98. Morse, D.R.: Immunologic aspects of pulpal-periapical diseases. Oral Surg 43:436-451, 1977.

99. Hassan, E.H.; Van Huysen, G.; and Gilmore, H.W.: Deep cavity preparation and the tooth pulp. J Prosthet Dent 16:751-755, 1966.

CURRICULUM VITAE

JAMES R. ROCHE

July 17, 1924	Born to Nelle and George Roche in Fortville, Indiana
1947	D.D.S., Indiana University School of Dentistry, Indianapolis, Indiana
1948	Forsyth Dental Infirmary for Children, Boston, Massachusetts, Intern Certificate
1949	James Whitcomb Riley Hospital for Children Dental Clinic, Indianapolis, Indiana, Indiana University, Teaching Fellowship
1949-52; 1954-68	Private practice of pedodontics, Indianapolis, Indiana
1949-52; 1954-68	Instructor of Pedodontics (Part-time), Assistant Professor of Pedodontics (Part-time), Indiana University School of Dentistry
1952-54	Captain, U.S. Army Hospital, U.S. Military Academy, West Point, New York
1968-Present	Professor of Pedodontics, Indiana University School of Dentistry
1969-76	Chairman, Division of Graduate Pedodontics, Indiana University School of Dentistry
1974-81	American Board of Pedodontics
1976-80	Assistant Dean for Faculty Development, Indiana University School of Dentistry
1980-Present	Associate Dean for Faculty Development, Indiana University School of Dentistry
1982-Present	Executive Secretary, American Board of Pedodontics

Professional Societies

American Dental Association
American Society of Dentistry for Children
American Academy of Pedodontics
Association of Pedodontics Diplomates
Pierre Fauchard Academy

ABSTRACT

EVALUATION OF A STERILE PULPOTOMY PROCEDURE

by

James R. Roche
Indiana University School of Dentistry
Indianapolis, Indiana

Pulpotomy procedures for the treatment of pulp exposure or disease have traditionally used an approach from the occlusal surface in primary molars, with a rotating bur or a spoon excavator being used to excise the pulp under clean conditions and with a medicament being applied to the amputation site.

The purpose of this study was: (1) to determine the feasibility of performing a sterile scalpel excision of coronal pulpal tissue and (2) to evaluate wound healing after a shield has been placed to prevent all substances from touching the blood clot at the amputation site.

Using sterile operating room procedures, four of five teeth in five dogs were rendered free of cultivable bacteria after antimicrobial treatments were applied. These findings replicated a preliminary <u>in vivo</u> experiment of 45 teeth which demonstrated that antimicrobial agents applied to tooth enamel in dogs were effective beyond the 0.001 level of significance, as compared to a control group. Furthermore, in the main study, negative cultures were obtained during 72 percent of the trials following each of the five major steps in the pulpotomy procedure. This included 80 percent negative cultures for bacteria after pulpal excision and blood clot formation, and 100 percent freedom from cultivable bacteria after a resin diaphragm was cemented to place.

In eight experimental teeth (three from the preliminary study and all five from the main study) the buccal dentin over the coronal portion of the pulp was removed by use of a tissue-protecting device without grossly macerating the pulpal tissue. In seven of these eight teeth, the coronal portion of the pulp tissue was amputated with a scalpel severance and pulpal biopsies were removed through the buccal preparation. In all eight teeth, the cavity preparation was accomplished without applying medicaments to the blood clot at the amputation site. A resin-stainless steel diaphragm was constructed under sterile conditions and secured in a buccal preparation without touching the pulpal amputation for a convalescence of 14-21 days, and this shield provided a buccal wall for closure with an amalgam restoration.

In one animal which had not received a previous autotransfusion for medical research, histologic sections of the pulp tissue in two root canals demonstrated satisfactory wound healing (a moderate and a mild inflammatory infiltrate was considered reversible). Three of the five dogs in the main study exhibited pulpal necrosis which was attributed to an interference in the immune system by previous autotransfusion research, and histologic evaluation of pulpal wound healing was therefore inconclusive.

This study demonstrates that the enamel surface can be rendered free of cultivable bacteria in the dog and the operative site can be maintained in this condition throughout pulpotomy, with the coronal portion of the pulp being removed without grossly macerating the pulp. Under these conditions there appears to be a potential for satisfactory wound healing, with possible implications for the clinical situation.